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Genetic and Biological Markers in Drug Abuse and Alcoholism

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Genetic and Biological Markers in Drug Abuse and Alcoholism

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Genetic and Biological Markers in Drug Abuse and Alcoholism

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Preface

Alcohol and drug abuse have become major health and social problems in the United States. Ten million American adults and 3.3 million youths in the 14 to 17 age range may be problem drinkers.(1) In a recent survey of high school seniors, nearly two-thirds of all seniors report illicit drug use at some time in their lives.(2)

A wealth of literature documents that genetic factors play a significant role in the transmission of at least some forms of alcohol abuse and alcoholism. Studies in humans and animals further suggest a genetic influence in individual variations of behavioral and biological responses, as well as differential organ sensitivities to the damaging effects of alcohol. The genetic influence in drug abuse is, however, less well documented. There are reports suggesting that genetics play a role in the user's performance after opiate use. Several studies suggest that preference for morphine is genetically related and that animal responses to cocaine and morphine also appear to vary with genetic background.

It is generally agreed that the genetic component in alcohol and drug abuse is multifactorial and there may be identifiable traits or markers that can single out individuals at risk. To foster research in this important area, the National Institute on Drug Abuse (NIDA) and the National Institute on Alcohol Abuse and Alcoholism (NIAAA) cosponsored a technical review entitled: "Genetic and Biological Markers in Drug Abuse and Alcoholism."

This meeting brought together researchers from the alcohol and drug abuse research communities to discuss the state of knowledge regarding genetic and biological markers, to identify research gaps, and to recommend future directions. Topics discussed represented a multidisciplinary approach ranging from family studies to animal models and from behavioral traits to gene mapping.

This monograph includes the 10 papers presented at this workshop and a summary of the presentations, prepared by Dr. Warren Nichols. Areas in need of additional efforts or further development and future directions for research recommended by workshop participants

are listed in a concluding chapter by the editors. The publication represents the beginning of a coordinated effort between NIDA and NIAAA to advance the field of genetic research in substance abuse. We hope that it will stimulate collaboration among scientists in various disciplines from both the alcoholism and the drug abuse research communities.

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Genetic and Biological Markers in Drug Abuse and Alcoholism: A Summary

Warren W. Nichols, M.D., Ph.D.

The workshop on "Genetic and Biological Markers in Drug Abuse and Alcoholism" reported in this monograph was jointly sponsored by the National Institute on Drug Abuse (NIDA) and the National Institute on Alcohol Abuse and Alcoholism (NIAAA). It brought together individuals from both abuse areas with expertise in clinical research, animal models, and molecular systems. The aims for examining genetic and biologic markers of substance abuse were twofold. The first aim was to develop methods for the use of markers that could predict individuals with a hereditary predisposition or susceptibility to alcoholism or drug addiction, as well as predict hereditary alterations that could enhance the toxic effects of these substances. The second aim was to examine the use of biologic and genetic parameters to detect damage or alterations produced by alcohol and drug abuse.

The difficulties encountered in these areas because of imprecision and variability of diagnostic criteria and classification were pointed out and discussed several times throughout the meeting. It was clear that bringing together interdisciplinary groups, such as those attending this workshop, was one good method of reducing this variability, and it was also clear that a series of separate meetings designed to unify diagnostic criteria and classification would benefit the entire area of drug and alcohol research. Further problems encountered are produced by the almost certain heterogeneity and multifactional nature of individuals abusing drugs and alcohol.

The markers or phenomena that may be of use in recognizing or detecting alcohol and drug abuse that were discussed at the meeting included: phenotypic and genotypic areas from behavioral observations and clinical genetic studies; polymorphisms in gene products, such as pharmacogenetic differences in metabolizing enzymes, isoenzyme variations, antigenic markers as blood groups, and the HLA systems; and DNA polymorphisms detected by restriction enzyme fragment length differences.

Strong evidence for a genetic factor or factors in alcoholism was presented in two clinical research papers by Drs. C. Robert Cloninger and Marc Schuckit. Both studies reported a marked increase in the prevalence of alcoholism in a population which had a first-degree relative that was alcoholic versus the general population. Various demographic means, including adoption studies, demonstrated that this was related to a genetic trait rather than to environmental exposure. However, both studies agreed that there were multiple genetic and environmental influences in this complex phenotype. Dr. Cloninger also reported a large excess of male over female alcoholics in both the general population and the population with first-degree alcoholic relatives. These differences appeared to have a genetic basis and could not be explained by socioenvironmental factors alone. Similar studies on opiates and other drugs of abuse are not currently available, but it is believed that from the alcohol experience similar studies could be established to determine the role of genetic factors in drug abuse.

Dr. Arthur Falek presented clinical research studies designed to examine opiate-induced alterations in chromosomes, DNA repair, and immunologic functions. Studies of peripheral lymphocytes of addicts demonstrated a significant increase in the levels of chromosome aberrations and sister chromatid exchange (SCE) events. There was also an increased response in these parameters after treatment with ultraviolet light. Formation of T lymphocytes E-rosettes was also reduced in these addicts. Some recovery of all parameters was observed in long-term methadone patients and marked individual variation occurred. The large number of variables in street drug users and the importance of separating effects of abuse substances from susceptibility to abuse substances was emphasized by Dr. Falek. He pointed out that there is at present no study that indicates a genetic potential to altered response to opiates, and that twins may be a suitable way to explore this hypothesis if a sufficiently large addict population is available for study.

Animal models were discussed in separate presentations by Drs. T.K. Li, Allan Collins, and Louis Shuster. Dr. Li described his studies on alcoholism using a rat model. By selecting and breeding rats that had a preference for alcohol over water, Dr. Li has developed animals that self-administer alcohol by mouth and achieve pharmacologically significant blood levels for the pharmacologic effect rather than for the caloric value, taste, or smell. The ethanol is positively reinforcing and leads to tolerance and physical dependence. Animals bred for these characteristics exhibit lower levels of serotonin and 5-hydroxyindolacetic acid in various areas of the brain. Other biochemical and physiologic parameters are being sought with the long-term goal of eventual human application.

Drs. Collins and Shuster discussed the use of various strains of mice in studies of alcohol and drug abuse. They pointed out that some markers of abuse substances that are of interest may require the introduction of the substance to initiate the marker. Also, important markers may be found in tissues not readily accessible in humans. In both situations, animal models may overcome these difficulties. The wealth of inbred strains, recombinant inbred strains, congenic lines, and selectively bred lines offer excellent starting material for analysis of genetic predispositions. Marked differences in responses to opiates, ethanol, and nicotine between C57Bl/6 and DBA/2 mice are being utilized to study differences in receptors, neurotransmitters, and endogenous opiates. Evidence was cited that high-affinity receptors are present for opiates but have not been found for ethanol. Evidence that animals can become addicted to endogenous opiates based on withdrawal symptoms after naloxone treatment was also described. Publication of a compendium of quantitative differences in drug responses of the many inbred lines and mutants as they are determined would be very helpful to investigators in this field.

Dr. Vesell's discussion of pharmacogenetic approaches to the prediction of drug response served as a bridge between the clinical studies, the animal models, and the molecular studies. He described genetic factors subject to environmental modifications that are capable of producing variations up to fortyfold in drug clearance, plasma drug half-life, and steady-state blood drug concentrations. Several hereditary conditions with unusual drug reactions based on pharmacogenetic differences were described, as were associations with phenotypic markers such as the HLA type. Dr. Vesell demonstrated mistakes in evaluating genetic metabolic polymorphisms in family studies if only the parent compound is evaluated in the absence of metabolic products.

Dr. Robert S. Sparkes listed the variety of polymorphic gene markers that are available for studying disease association and genetic linkage. Association refers to a close relationship between a trait or a disease and a specific allelic variant revealed by the study of unrelated individuals, and it is probably important in the pathogenesis of the disease or trait. Genetic linkage refers to the physical location of genes close to each other on the same chromosome and requires family studies for elucidation. Linkage studies can be used to determine which members of an at-risk family possess a gene which has been proven to be linked to increased susceptibility to a disease state, while association studies may be important in determining whether a specific genetic variant is associated with a reaction to alcohol or another drug. Of the various polymorphic gene markers available, Dr. Sparkes pointed out the several advantages of DNA restriction fragment length polymorphisms (RFLP). These include the fact that the gene or genes in the polymorphic DNA fragments need not be expressed or even identified, and that all

regions of the genome can be covered rather than the approximately 20% that occurs with other phenotypic markers. This means that any gene under question should be linkable.

Dr. Moyra Smith-Wright described the preparation of a cDNA clone for the alcohol dehydrogenase 1 gene (ADH1) from messenger RNA for the polypeptide gene product. This probe was used to study the closely homologous three class I ADH genes for polymorphism at the DNA level. The results suggest that polymorphisms occur in at least two of the ADH genes and that the polymorphisms are heritable. By the use of somatic cell hybrids, it has also been possible to map these genes to the long arm of chromosome 4. There are indications that the fetal alcohol syndrome has genetic factors that may be approachable through these polymorphisms in genes coding for metabolic enzymes and that this may be a good starting point for work with these probes.

All in all, this workshop was very productive with excellent interaction between the various interdisciplinary groups. It is certainly time, and this is an excellent method to select well-thought-out studies in new areas that may contribute to both prevention and treatment of these large problems.

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Polymorphic Gene Marker Studies

Robert S. Sparkes, M.D.

INTRODUCTION

Common genetic variations are called polymorphisms and refer to genetic traits for which at least two allelic variants occur with a frequency which cannot be attributed to recurrent mutation alone; therefore, each variant usually occurs with a frequency greater than 2%. Man, being a diploid organism, has only two alleles at each locus, but in a population of individuals there may be many alleles at a locus. The biological significance of many of these polymorphisms remains to be determined.

Currently available polymorphic genetic markers can be grouped into phenotypic and DNA markers. The phenotypic markers include red blood cell antigens, red blood cell isoenzymes, serum proteins, and HLA antigens. The polymorphic variants at the DNA level are called restriction fragment length polymorphisms (RFLPs).

APPLICATIONS OF POLYMORPHIC GENE MARKERS

Polymorphic genetic variation has applications in a number of areas, but basically they afford means to identify or distinguish the presence or absence of specific genetic alleles. This variation can be used for individual identification, such as in forensic studies where blood stains are compared with fresh blood from a known person. The oldest application of genetic polymorphic studies in the laboratory is with blood transfusions in which there is matching between donor and recipient for polymorphic red blood cell antigens, especially in the ABO and the Rh systems. Similar application is made in tissue transplantation using the HLA polymorphic variation. In bone marrow transplantation, the other polymorphic markers, such as isoenzymes, can be used to identify the presence of donor cells in a recipient following a transplant; this is based upon differences in any of the polymorphic markers between donor and recipient. These polymorphisms are also used for paternity evaluation in which these markers are compared between the child, its mother, and the putative father, searching for evidence that there is a genetic trait that the child could not have received from the man in question. Such information is also needed in genetic

linkage studies (see below). Isoenzyme polymorphisms have been used to demonstrate cross-contamination of tissue culture cell lines. It is not uncommon to find a mixup in cultures and that the investigator is not working with the expected culture; isoenzyme studies have demonstrated that upwards of 30% of such cultures are of an unexpected type. Major applications have also been made to the study of human gene mapping using polymorphic traits in linkage analysis. Finally, studies have been made to evaluate possible associations of diseases or other traits with specific allelic types, particularly with the ABO blood group and the HLA white blood cell system.

GENETIC LINKAGE

Human gene mapping has made major advances in recent years. This has largely been achieved through the use of interspecific somatic cell hybridization. This approach is based upon the nonrandom loss of human chromosomes from the somatic cell hybrids formed between established rodent cell lines and human cells. A correlation is made between which human genes and which human chromosomes are present in a panel of different hybrid clones. Although each clone usually has more than one human chromosome, the different clones are chosen so that the total panel will permit assignment of a specific gene to a specific chromosome. This technique can also be used to regionally map genes on a chromosome by starting with human cells which contain a chromosome rearrangement, usually a translocation. The correlation is then made between the gene in question and that part of a chromosome which remains in the somatic cell hybrid. One of the advantages of this technique is that it does not depend upon polymorphism for the gene which is being mapped. Much of the past work with somatic cell hybrids has utilized species differences in enzymes which can be seen on electrophoresis or isoelectric focusing.

In contrast to the somatic cell hybridization approach, human gene mapping through linkage analysis does depend on genetic polymorphism. With genetic linkage, one measures gene map distance between two genes as reflected in the number of cross-overs which occur in meiosis between the two genes (Conneally and Rivas 1980). Linkage is the tendency of genes on the same chromosomes to segregate together, i.e., linked genes are transmitted to the same gamete more than 50% of the time. The closer genes are to each other, the more frequently they will be transmitted together. On the other hand, genes that are far apart on some of the larger chromosomes may segregate randomly in relation to each other, even though they are on the same chromosome. All genes that are located on the same chromosome are said to be syntenic. While all genes that are linked are syntenic, the converse may not be true. Linkage analysis requires the study of families. Generally, families in which a genetic trait of interest occurs are examined. Until recently, most linkage studies have used the phenotypic polymorphic traits of the red blood cell antigens, red blood cell isoenzymes, serum proteins, and HLA variants. Through the use of statistical analysis, lod scores can be obtained and these reflect the likelihood that two genes are linked to each other and how close they may be. Because of the relative limited number of polymorphic phenotypic markers available and because these cover only a fraction

of the total human genome (about 20%), genetic linkage analysis has not been highly successful. However, with the introduction of the DNA RFLPs, a new era has arrived in human gene mapping and, in theory, it is now possible to map any human gene through this approach.

All genetic variation reflects differences at the DNA level. This is true for the phenotypic markers which reflect genetic changes based upon differences between alleles that can be determined by electrophoretic techniques, immunological techniques, or other biochemical methods. With the RFLPs of DNA, one detects these differences directly in the DNA. Study of these polymorphisms is based upon the following. Restriction endonucleases split DNA at specific base sequences. These base sequences at the same site in the DNA may vary between persons. With the use of different restriction endonucleases, potentially a large number of different base sequences may be detected in a population of individuals. By isolating DNA from cells of individuals and treating the DNA with restriction endonucleases, one can separate the DNA fragments by molecular size using agarose gel electrophoresis. One then hybridizes radioactively labeled DNA probes of specific function or DNA probes of nonspecific or unidentified functions (anonymous probes) to the DNA which has been treated with the restriction endonucleases and separated by the electrophoresis. Differences in the size of the DNA segments can be detected with the probes. These differences can then be treated by the standard linkage approach described above. The DNA RFLPs have a number of advantages (table 1). With the availability of a large number of restriction endonucleases, it is very likely that each and every gene will be found to demonstrate polymorphisms following treatment with one or more of these restriction endonucleases. It has been estimated that perhaps only 165 DNA probes could span or map the human genome (Botstein et al. 1980). This would be true if the probes were mapped at random. However, it has been estimated that many more markers may have to be studied and evaluated in order to find the minimum number which would be randomly distributed over the genome (Lange and Boehnke 1982). Nevertheless, it appears at this time that with sufficient effort the appropriate probes can be isolated and used to eventually map the whole human genome. It should be noted that these DNA probes can also be used in conjunction with the somatic cell hybridization approach referred to above. In addition, they can be used in a new approach called in situ hybridization in which the radioactive probe is hybridized directly to chromosomes on a microscope slide.

Many human genes are being rapidly mapped through the use of the DNA probes using these three methods: linkage, somatic cell hybridization, and in situ hybridization. For example, one prominent example using this recombinant DNA approach has been the demonstration of linkage of the gene for Huntington's disease to an anonymous gene identified by the DNA probe, G8 (Gusella et al. 1983). Although still not applicable at the clinical level, these developments offer the prospect in the near future of being able to enhance genetic counseling for families in which Huntington's disease occurs. Poten-

tially, such an approach could be used to make prenatal diagnosis to identify persons at risk not only before the symptoms start, but even before the individual is born.

TABLE 1

Advantages of DNA RFLPs

1. Gene or DNA sequence need not be expressed;
2. RFLPs can be found in all regions of the genome;
3. Probably all genes or DNA sequences can be "made" polymorphic with use of different restriction endonucleases;
4. Same technique is used for all markers;
5. May lead to isolation of a gene of interest; and
6. Any gene should be "linkable."

ASSOCIATION

Genetic linkage and association are terms that are often used interchangeably, but they describe different phenomena with different implications (Vogel and Motulsky 1979; Hodge and Spence 1981). However, within a single family it may not be possible to distinguish linkage from association. Evaluation of each requires the use of genetic polymorphisms, which may in part contribute to the confusion. Linkage relates to genetic loci, and the alleles at these loci are useful only as markers for the loci. Evaluation of linkage requires the study of families, especially large families. Association refers to a concurrence greater than predicted by chance between a specific allele at a locus and another trait which may or may not have an obvious genetic basis. Evaluation of association requires the study of unrelated individuals. Association may give an insight into susceptibility and pathogenesis of a trait or disease. Thus, association studies may be used to identify a genetic factor in a disease. With the exception of linkage disequilibrium, association is not due to linkage. Association may result from: pleiotrophic effects of a single gene; epistatic interaction; selection in relation to environmental factors (for example, sickle cell gene and malarial resistance) and population stratification.

The early examples of association are related to the ABO blood groups, because this was the earliest polymorphic trait available for study. Examples of significant associations between the ABO blood groups and diseases include a higher frequency of blood type A in persons with neoplasia of the intestinal tract, cervix, and breast, while there is increased frequency of blood type O in persons with duodenal and gastric ulcers. However, these associations are not sufficiently

strong to have clinical importance. When the highly polymorphic HLA system became available, it was applied to a large number of diseases in a search for associations. Some of the significant associations between HLA and diseases include type B27 with ankylosing spondylitis and Reiter's disease, and type DR3/4 with insulin-dependent diabetes mellitus.

In addition to the association of the sickle cell gene and the resistance to malaria noted above, a similar resistance has been noted for G6PD deficiency and malaria. Another association that has been described in relation to malaria is the Duffy blood group system in which individuals of the Fy(a-b-) phenotype demonstrate resistance to malaria. Evidence has been presented that the Duffy a and b antigens may act as red cell receptors for malaria parasites. As expected, the genomic types for the resistance to malaria have been found to occur with a high frequency in malarial areas. These are also nice examples of how environmental factors may contribute to specific allelic frequencies.

Demonstration of associations may help to: identify a genetic component to a disease; clarify pathogenesis of a disease; facilitate preclinical testing to identify persons at risk so that appropriate counsel may be given; and recognize disease heterogeneity.

Thus, associations may be important in determining whether a specific genetic variant is associated with reaction to a drug or alcoholism. On the other hand, genetic linkage may be useful to identify a gene or genes which may be important in determining reactions to a drug or alcohol, but whose function is not known. From linkage to a testable polymorphic gene marker, one may be able to identify who in a family may be at risk to have a gene for drug abuse or alcoholism.

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Individual Differences in Opiate-Induced Alterations at the Cytogenetic, DNA Repair, and Immunologic Levels: Opportunity for Genetic Assessment

Arthur Falek, Ph.D.; John J. Madden, Ph.D.; David A. Shafer, Ph.D.; and Robert M. Donahoe, Ph.D.

In humans there are several strategies to identify the significance of hereditary factors underlying the occurrence of a behavioral and/or physical trait, including: a) family studies, b) twin studies, c) linkage studies to a genetic marker, and d) adoption studies. In particular, where expression of a trait may be influenced by sociocultural factors, consistent findings are required from several strategies to support the importance of genetic factors. For example, family, twin, genetic marker and adoption studies (Goodwin 1981) have provided consistent support for the importance of a genetic component for alcoholism.

Linkage studies of specific traits with genetic markers have recently been expanded from linkage by blood groups, HLA haplotypes, and other such chromosomally located protein markers to those linked by specific DNA probes such as the recently announced linkage between a polymorphic DNA probe on the short arm of chromosome 4 and Huntington's disease (Gusella et al. 1983; Vesell 1984). This new approach is limited at present to the study of clearly identified single gene inherited disorders but may in the future be appropriate for studies of apparent polygenetic traits with strong environmental components such as alcoholism.

While genetic studies employing different strategies have demonstrated the importance of the genetic component in alcoholism, unfortunately no such self-consistent data have been obtained for opiate abuse. As reported by Liston and associates (Liston et al. 1981) and reaffirmed most recently in our search of the drug abuse literature, there is a paucity of information on the contribution made by genetic factors to individual differences in response to opiates among humans. On the other hand, as noted by Liston and associates, there have been studies of genetic influences on performance after opiate use as well as on preference in animals for morphine. These studies demonstrated that there were significant differences in locomotor

activity among different strains of rats following morphine administration (Eidelberg et al. 1975). They also found that rats could be bred with a preference for morphine (Nichols and Hsiao 1967) and that mice inbred for alcohol use also preferred morphine (Eriksson and Kiianmaa 1971). These animal studies demonstrating genetic mechanisms underlying variability in response to morphine are reviewed by Dr. Shuster (this volume).

In humans, the physiological (cold pressor test) and psychological parameters of responses to pain and to morphine in 10 sets of normal monozygotic male twins were measured (Liston et al. 1981). This study (from the VA Hospital in Los Angeles) provided no evidence for a genetic component in response to pain (either pain threshold or pain tolerance) before or after administration of morphine. Although these investigators did find significant twin pair effects on two measures of anxiety, they indicated that the number of twin sets in their study was too small to produce significant differences between pairs as compared with differences between the twin partners. The only other report in the literature on genetic aspects of opiates in humans is on one pair of monozygotic twins discordant for heroin abuse (Grumet 1983).

Our own study of cellular genetic aspects of opiate abuse was initiated to determine whether there was an impact on the genome among humans using opiates. Initial studies were conducted to examine levels of chromosome damage (CD) among opiate addicts as compared with controls, since an increased level of such chromosome alterations implies DNA damage and often may be correlated with an increase in mutational events. For the first study, we compared leukocyte chromosomes of 16 addicts with 16 controls (Falek et al. 1972). Our findings revealed 32 random chromosome aberrations among the 16 drug patients for a rate of 2.7% (calculated in the first study as the number of aberrations found divided by the number of metaphase chromosome complements scanned) as compared with 4 chromosome breaks in the leukocytes of the 16 control subjects for a rate of 0.4%. We consistently found a frequency of 0.4% chromosome damage among normal populations during the time we were conducting these studies. There were some opiate addicts who showed no evidence of chromosome damage and obviously there were others who displayed more than 2 damaged chromosomes to provide the 32 damaged chromosomes among the almost 1,200 metaphase complements analyzed. There was no correlation of the chromosome damage level in the addicts with the length of time of drug use, the type of street opiate, or the abuse of other drugs. Further studies in which addicts were followed longitudinally for a period of more than 1 year through methadone treatment or nonchemical detoxification provided evidence that the increased number of damaged chromosomes at time of admission also reflected a significantly increased frequency of cells with at least one damaged chromosome (Falek and McFadden 1973; Falek and Hollingsworth 1980). Inter- and intra-individual variability was clearly evident from one time point to the next (table 1).

After a year the CD levels were reduced to about 0.6% for those remaining in the study, which was not significantly different from the 0.4% found for normal controls. Evidence that the increase in CD was opiate induced rather than a result of other environmental factors was provided in cytogenetic studies in which street opiates or morphine were given to rhesus monkeys (Fischman et al. 1983). As with the human addicts, there was a significant increase in CD in the opiate treated monkeys as compared with controls, proving that the CD increase was not merely ascribable to the human addict milieu.

TABLE 1

INDIVIDUAL VARIABILITY IN DRUG ADDICTS FOLLOWED FOR 8 WEEKS IN METHADONE TREATMENT

Cells with Chromosome Breakage

Cases	Pre-Methadone	24 hours Post-Methadone	8 weeks Methadone Maintenance
1	1	1	5
2	4	1	1
3	1	0	1
4	1	1	1
5	1	6	2
6	5	0	0
7	0	1	3
8	0	6	2
9	2	6	5
10	3	6	2

A second method of examining the question of chromosome damage is by the study of sister chromatid exchange frequencies (SCE). SCE and CD are known to be the products of independent mechanisms that result from different types or conditions of DNA damage (Galloway and Wolff 1979; Lin and Wertelecki 1982). SCE occurs with a much higher frequency than CD, and such analysis has become established as a rapid, sensitive, and quantitative assay to determine genetic damage (Shafer 1982). It is evident from the literature that: 1) there are individual differences in baseline frequency among people (Crossen 1982); 2) there is a hereditary component in SCE formation indicated by significant sib-sib and parent-child, but not parent-parent, correlations in SCE base levels (Cohen et al. 1982); 3) there are increased SCE base levels or specific SCE sensitivities in such genetic diseases as Bloom's syndrome, xeroderma pigmentosum, or Fanconi's anemia (Ray and German 1982); 4) there are significantly elevated rates of SCE after *in vivo* exposure to

known mutagens (Hansteen 1982); and 5) increased SCE induction in vivo is significantly correlated with later carcinogenesis or transformation (Popescu and Dipaolo 1982; Raposa 1984). As to when such effects may occur in the cell cycle, SCE can only be induced by treatments prior to completion of S phase replication, while CD can also be induced by G_2 treatments with mutagens such as X-ray and radiomimetic agents.

Figure 1 is a histogram of SCE base levels for 47 heroin addicts and 39 controls (Shafer et al. 1983). There were differences in base level counts among individuals scored as SCEs/cell in the chromosomes of about 15 metaphase cells per individual. In the opiate addict population, the mean ranged from approximately 8.5 to 20.5 with an overall mean of 13.82 ± 4.76 SCE/cell. For controls, individual mean SCE scores ranged from 5.5 to 15.2 with an overall mean score for controls of 11.16 ± 3.97. Analysis of variance indicates a highly significant difference between the opiate and control groups ($F = 22.00$, $p \leq 0.001$).

Individual differences were observed in the frequency of SCE not only when opiate addicts were compared with controls at base levels, but also when the lymphocytes from both populations were exposed to far ultraviolet light (far UV) at G_1 and S in the cell cycle. Figure 2 shows a histogram of SCE counts at base level as well as after exposure to far UV ($14 J/m^2$ with Morowitz correction) at G_1 (18 hours before harvest) and at S (12 hours before harvest). When base level and UV induced SCE frequencies are compared for individual subjects, the data demonstrate that interindividual variability plays a significant role in SCE responses to far UV stress. Analysis of variance with repeated measures (to simultaneously and separately compared SCE means at base level and at the two treatment points for each individual) as well as use of the nonparametric Mann-Whitney U test confirmed that the SCE frequencies among heroin addicts were significantly higher than controls and that the increase at base level was paralleled by increases of comparable magnitude with UV treatments at these time points.

Our studies on DNA repair synthesis (DRS) induced by far UV likewise demonstrate individual response differences (Madden et al. 1979). DRS is defined as a mutagen induced increase in the incorporation of thymidine into all precipitable cellular DNA beyond that incorporated by semiconservative replication. DRS is presumptively an indicator of DNA excision repair and as such has been used to identify such genetic defects as xeroderma pigmentosum (Cleaver and Bootsma 1975). We, therefore, investigated repair of far UV damage to determine whether there was etiologic evidence to explain the observed increase in chromosome damage among opiate addicts and the decrease in such damage in many long-term methadone patients.

DNA repair synthesis fluence curves for different subjects generally show a linear increase in thymidine incorporation with increases in far UV doses up to 15 to 20 J/m^2, but a few

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individuals reach maximal incorporation at <5 J/m^2 (poor repairers), while others are saturated only at doses of >50 J/m^2 (super repairers). The repair capacity is defined as the plateau level of the curve (i.e., the maximum measurable thymidine incorporated). The control subjects demonstrated a marked individual response to far UV, with a mean value of 14,700 dpm/A_{260} and a standard deviation of $\pm$9,700 dpm/A_{160}. This result supported the hypothesis of significant variability in the capacity of leukocytes of individuals in the general population to handle fluences of far UV. Variables such as age, sex, or alcohol use did not significantly contribute to the variance, but tobacco use significantly lowered the mean DRS score to 10,300 dpm/A_{260}, primarily as a result of the loss of high repairers from the group. In contrast, heroin addiction produced an even greater decrease in the mean of far UV induced repair in comparison to controls as evidenced by a shift in the maximal DRS response to largely the poor and low median portion of the curve (X = 4,300 dpm/A_{260} $\pm$ 4,200). After long-term methadone treatment (X = 4.8 years), there was no significant difference between patients in treatment and controls (X = 13,200 dpm/A_{260} $\pm$ 9,300). These data imply a correlation between DNA repair and chromosome damage levels. When DNA repair level is depressed, there is an increased frequency in cytogenetic effects, while the cytogenetic damage is depressed when DNA repair returns to more normal levels.

Based on the cytogenetic and DNA repair findings, we conducted immunologic explorations to more specifically define the leukocyte cell type or types involved in these repair and cytogenetic changes (McDonough et al. 1980, 1981; Donahoe et al. 1985a). While there were no significant changes observed in B lymphocytes or total white blood cell count, the T cell values decreased significantly from approximately 69% found in the control population to about 33% in the addict population (figure 3). The addicts in the study were on street heroin almost exclusively for approximately 10 years. For the long-term methadone patients, the E rosette frequency was increased to 56.7%. It is clear in both the addict and methadone populations that there is a great diversity in individual response. When viewed over time, as seen in figure 4, the data show that on the average the E rosette depression becomes more severe the longer the addict uses street opiates, but, as seen in the outliers, this does not totally account for the variability in rosetting. Similarly, individual differences are displayed in figure 5 regarding the effect of methadone therapy on the percent of circulating E-rosette forming lymphocytes. This somewhat unpredictable response to opiates and therapy was also reported by Brown and colleagues (1974) in their study of mitogen induced growth of lymphocytes from patients followed longitudinally during methadone therapy.

Kinetic assay studies to assess opiate effects on rates of T cell E-rosetting indicate that a major biological effect of the opiates relates to their ability to modulate expression of

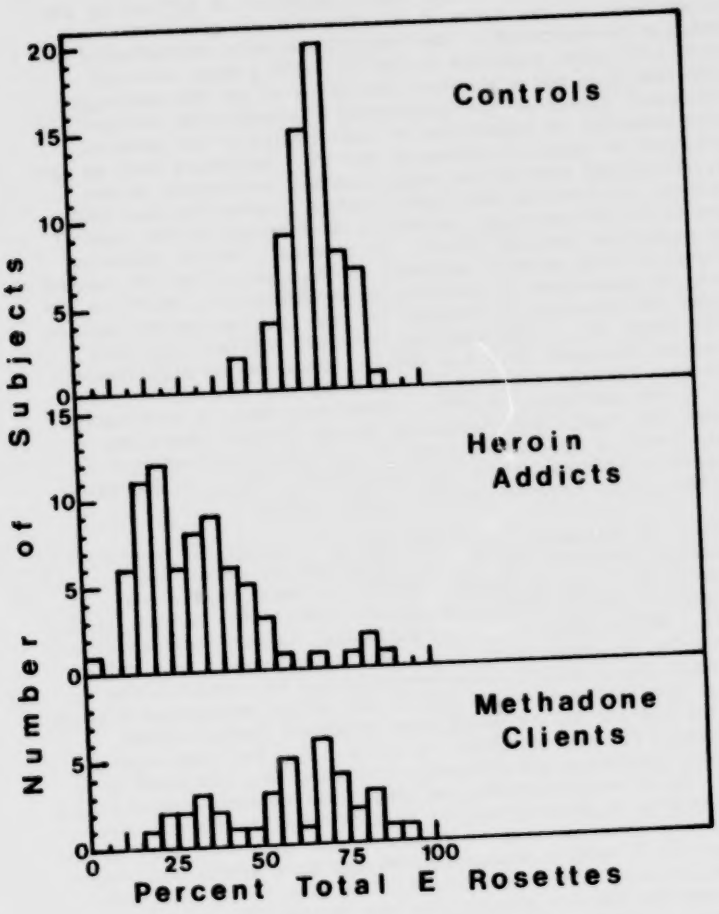

FIGURE 3

Histogram of the percent sheep erythrocyte (E) rosettes formed by lymphocytes from control subjects, heroin addicts, and methadone clients.

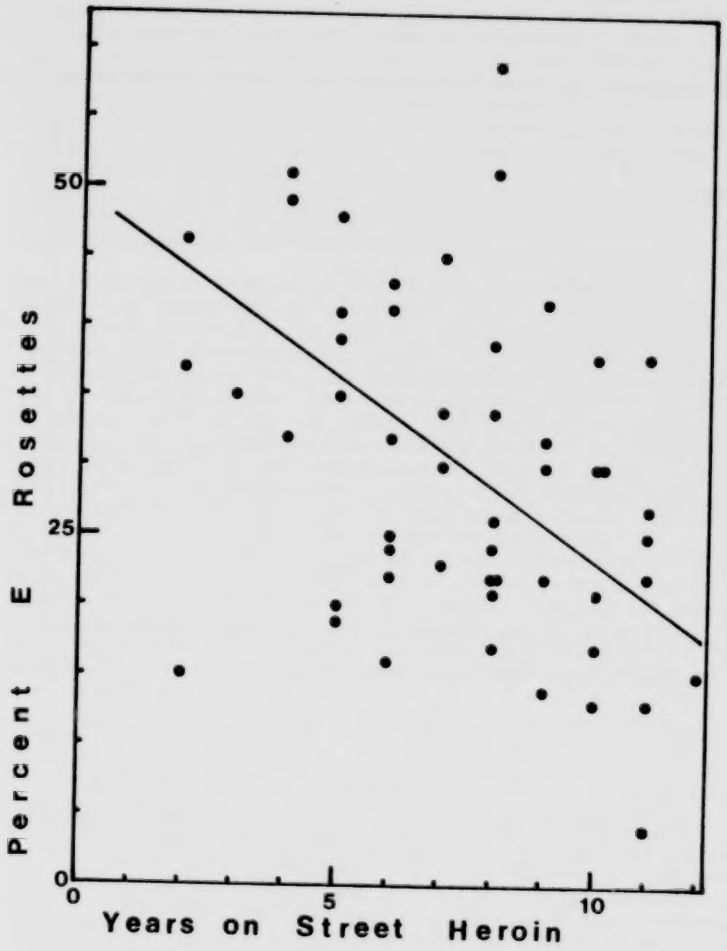

FIGURE 4

The effect of years of street heroin use on the percent E rosettes formed by lymphocytes from addicts. The line shown represents the best regression line which can be fitted to the data.

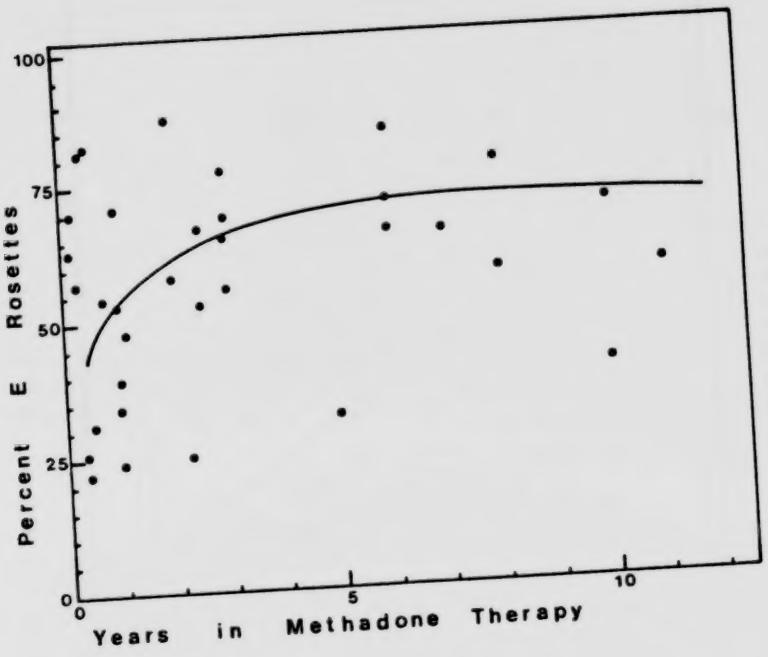

FIGURE 5

The effect of years of methadone use on percent E rosettes formed by lymphocytes from methadone clients. The great variability seen can arise both from variability in the previous street drug history of the client (type of opiate, average daily dose, length of time addicted, individual response to street heroin) and from the individual response of the client to the methadone. While there is a general return to control percents of E rosettes, a few subjects show pronounced rosette depressions even after years in therapy. Because of the rigorous screening of these clients for illicit drug use during this period, these outliers cannot be ascribed to illicit drug use.

regulatory proteins on the T cell membrane (Madden et al. 1983). We also found that opiate addiction associates with altered expression of T cell antigenic markers which may occur through opiate modulated enhancement of suppressor T cells, resulting in a depression of Th/Ts ratio in long-term addicts (Donahoe et al. 1985b). The observations that opiates have the ability to modulate surface proteins/receptors on T cells support the possibility that T cell constituencies among heroin addicts are one of the bases for individual variation in the expression of cytogenetic and DNA damage seen in our addict populations. With the ability to secure relatively pure T cell populations, it is now possible to investigate the genetic aspects of such potential differences.

Our cytogenetic, biochemical, and immunologic assays provide evidence of significant alterations in users of street opiates as compared to controls, as well as individual differences in response to street opiates and individual levels of amelioration of these alterations over time with methadone. As indicated in the introduction to this chapter, one method of genetic analysis in humans, particularly with assays that apparently are not directly under sociocultural influences, is based on concordance rates in monozygotic (MZ) and dizygotic (DZ) twin partners. Monozygotic twins are genetically alike while dizygotic twins are no more similar than pairs of siblings in a family. In his presidential address delivered before the Society for Psychophysiological Research, David Lykken (1982) related that human geneticists have concentrated on two basic mechanisms of genetic transmission. He noted that mendelian mechanisms depend on the presence or absence of a particular allele at a specific gene locus, while the galtonian mechanisms are concerned with polygenic transmission in which the value of a metrical trait such as height is apparently determined by the additive action of a number of genes at different loci. Dominance (between alleles at one locus), simple epistasis (a type of dominance between two genes at different loci), and polygene epistasis (a nonadditive configuration resulting from interactions among more than two independent or partly independent genes) may modify polygenic transmission. In particular, the latter, while genetic, may not run in a simple manner in families. To determine whether or not such complications occur in responses to and predilection for opiate use in humans, comparative twin differences with regard to protein modulation or receptor number on the T cell membrane between concordant and discordant MZ and DZ twins may be a most fruitful approach to an examination of the genetic aspects of this disorder.

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Pharmacogenetic Approaches to the Prediction of Drug Response

Elliot S. Vesell, M.D.

As the chapters in this monograph illustrate, numerous genetic and environmental factors can influence the metabolism and effects of ethanol. Despite this recent progress in identifying these factors, the underlying cause or pathogenesis of alcoholism and of abuse of other substances remains illusive and ill defined.

It follows that the primary products of putative "causative" genes are unknown. The genetic and environmental factors thus far investigated appear to be peripheral rather than primary with respect to causation of the underlying behavioral aberration associated with drug abuse. So imprecise are our present concepts of specific genetic causes of drug abuse that recommendations for research in this area are hazardous and difficult to justify if strict criteria are applied.

PHARMACOGENETICS

Pharmacogenetics is defined as the study of specific genetic factors that cause large interindividual variations in response to some drugs. Pharmacogenetic investigations would not be likely to reveal the pathogenesis of substance abuse, but rather might explain why certain subjects who abuse alcohol and other drugs may be particularly susceptible to injury and toxicity, whereas others who take larger doses resist damage and do not develop the disease (Kalow 1962; Motulsky 1964; LaDu 1972; Vesell 1973; Kalow 1982; Propping 1978). For example, racial differences in an isozyme of aldehyde hydrogenase explain the greater susceptibility of Oriental subjects to such effects of ethanol as facial flushing; this example illustrates the capability of pharmacogenetics to elucidate the basis of interindividual variations in susceptibility to drug toxicity and was discovered by a distinguished investigator in pharmacogenetics, H. Werner Goedde (Goedde et al. 1979, 1984; Harada et al. 1980; Agarwal et al. 1981). Another more controversial example of pharmacogenetic progress in this area is the possible association of the immunogenetic markers HLA-B40 and DRW9 with alcoholic cirrhosis of the liver in Japanese subjects (Miyamoto et al. 1983). Although certain HLA haplotypes are highly associated with several disorders of a fundamentally immunologic nature, such

as ankylosing spondylitis, lupus erythematosus, and rheumatoid arthritis, several previous studies failed to observe such associations of HLA haplotypes with alcoholic cirrhosis, a disease not generally considered to be due to an immunologic abnormality. However, recently, several immunologic abnormalities have been described in patients with alcoholic cirrhosis (Sorrell and Leevy 1972; Tannenbaum et al. 1969; Tsuchimoto 1982).

In the area of abuse of drugs other than alcohol, no specific pharmacogenetic entities have thus far been identified. However, several observations suggest that pharmacogenetic mechanisms might be operative and might contribute to large interindividual variations already documented to occur in the metabolism of methadone (Kreek 1973) and possibly in the metabolism of cocaine and heroin, two other commonly abused drugs whose elimination from the body also occurs mainly through biotransformation (Goodman and Gilman 1980).

In human subjects, pharmacokinetic values are more conveniently and reliably measured than pharmacodynamic ones. Pharmacodynamic variations undoubtedly also exist, and some of these may be maintained by genetic mechanisms. However, it is not yet feasible to obtain precise pharmacodynamic measurements noninvasively in human subjects. Much technical progress is necessary before pharmacodynamic measurements can be gathered in humans reliably, safely, and noninvasively.

The following review of pharmacogenetic progress and methodology is offered to stimulate and suggest analogous studies on drugs of abuse. It is readily acknowledged that formidible methodological problems are posed by adapting to drugs of abuse these pharmacogenetic approaches based on the administration of single safe doses of various prescription drugs to normal subjects under carefully controlled environmental conditions. Results of similarly designed studies on drugs of abuse in addicts might be uninterpretable because of confounding by numerous environmental perturbations, including the smoking of cigarettes and/or marijuana, nutritional variations, and intake of other drugs such as ethanol. Ethical considerations render objectionable the administration to unaddicted subjects of drugs at dosage levels usually ingested by drug abusers. Other approaches would have to be taken in such normal subjects. Possibilities include administration of tracer doses of ^{14}C- or ^{13}C-labeled drugs or growth of normal cells in culture to investigate their pharmacokinetic and/or pharmacodynamic responses to various drugs of abuse.

DYNAMIC INTERACTIONS AMONG THE GENETIC AND ENVIRONMENTAL FACTORS THAT MODIFY DRUG RESPONSE

Unlike the numerous problems that beset studies to identify factors that cause pharmacokinetic variations among subjects after administration of drugs of abuse, much progress has been achieved in investigating such factors that influence the disposition of prescribed drugs eliminated primarily through hepatic metabolism. This progress has occurred largely through availability of normal

human subjects who can safely ingest single doses of various model drugs, such as antipyrine, aminopyrine, amobarbital, debrisoquine, sparteine, mephenytoin, acetaminophen, theophylline, etc. Antipyrine has proven particularly fruitful in disclosing numerous environmental factors that can interact with each other as well as with genetic factors to determine antipyrine elimination rates and plasma antipyrine concentrations. The design of figure 1 suggests dynamic interaction of such factors. In contrast to addicts, the normal subjects used to identify the factors shown in figure 1 were all carefully defined and uniform with respect to almost every factor in the outer circle. This standardization of prevailing environmental conditions among the subjects permitted each variable to be investigated independently of all others. Dose-response curves could be generated, relating increments of a specific factor to subsequent changes in antipyrine disposition. This model drug approach is exceedingly sensitive in detecting factors that can affect drug disposition, mainly because each subject serves as his or her own control (Vesell 1979). Use of subjects as their own control eliminates extraneous contributions from many genetic and environmental perturbations that occur in the alternative experimental design where the control and experimental subjects differ, forming two separate groups that are compared. Nevertheless, like all laboratory tests, the model drug approach has limitations. Occasionally, it has disappointed some investigators whose expectations from it were unrealistic or unwarranted (Vesell 1979).

The main limitation as well as advantage of the model drug approach depends directly on the pharmacologic characteristics of the drug selected for administration. With respect to antipyrine, the principal advantage is that antipyrine decay in saliva and plasma reflects directly antipyrine metabolism in the liver by three separate cytochrome P-450 mediated monooxygenases. Since antipyrine is absorbed completely from the gastrointestinal tract, distributes in "total body water," is not bound significantly to tissue or plasma proteins, and is not eliminated to an appreciable extent by renal mechanisms, antipyrine decay in plasma and in saliva sensitively reflects its rate of hepatic biotransformation. Furthermore, since antipyrine is a drug with low hepatic extraction, its elimination is independent of hepatic blood flow. No other model drug yet proposed enjoys so many advantages as an indicator of hepatic drug-metabolizing capacity. Nevertheless, antipyrine elimination can only reflect the activities of a few of the many hepatic drug-metabolizing enzymes. Other such enzymes not directly involved in antipyrine biotransformation may be influenced to a greater or lesser extent than the antipyrine-metabolizing enzymes by a particular genetic or environmental factor. Hence, these effects may not be accurately disclosed with antipyrine. For example, the metabolism of debrisoquine and sparteine occurs through a different cytochrome P-450 mediated monooxygenase. For this reason, if many hepatic drug-metabolizing enzymes are to be investigated simultaneously, several model drugs in addition to antipyrine need to be used.

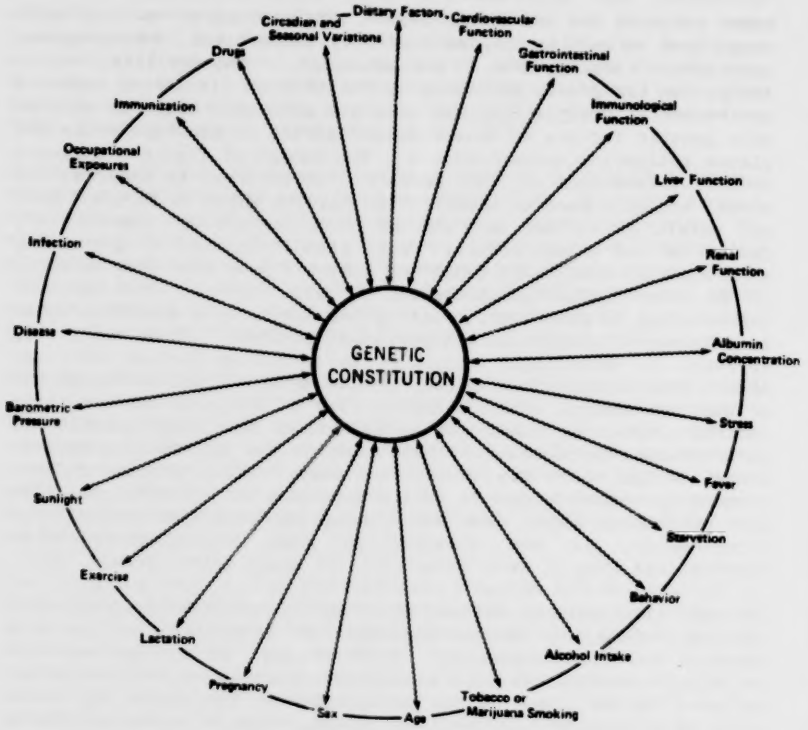

FIGURE 1

This circular design suggests the multiplicity of either well-established or suspected host factors that may influence drug response in man. A line joins all such factors in the outer circle to indicate their close interrelationship. Arrows from each factor in the outer circle are wavy to indicate that effects of each host factor on drug response may occur at multiple sites and through different processes that include drug absorption, distribution, metabolism, excretion, receptor action, and combinations thereof.

PHARMACOGENETICS: GENE-DRUG INTERACTIONS THAT PRODUCE DISEASE

Pharmacogenetics, the study of genetically determined variations in drug response, focuses on interindividual variations because they are frequently encountered and often present clinical problems. For example, the extent of these intersubject variations in response can range from threefold to fortyfold, depending on the particular drug and population. For drugs with low therapeutic indices, each patient's dosage regimen needs to be carefully adjusted to avoid drug toxicity or undertreatment.

Since many drugs are plant alkaloids, it is not surprising that genetic variations also exist in response to some dietary constituents and that some inborn errors of metabolism are worsened by dietary constituents. For example, galactosemia, phenylketonuria, Wilson's disease, celiac disease, and lactose intolerance due to lactase deficiency are exacerbated by foods containing galactose, phenylalanine, copper, gluten, and milk, respectively. Also, in several other inborn errors of intermediary metabolism, crises can be precipitated by certain drugs. For example, in diabetes mellitus, gout, and several forms of porphyria, administration of adrenal glucocorticoids, thiazide diuretics, and barbiturates, respectively, should be avoided.

Another group of inborn errors of metabolism exists in which the aberrant (mutant) protein participates directly, rather than indirectly, in drug disposition or action. These entities, the main focus in the past of the field of pharmacogenetics, include several rare monogenically transmitted conditions (acatalasia, atypical plasma cholinesterase, phenytoin sensitivity, and warfarin resistance) as well as some commonly occurring monogenically transmitted conditions (slow acetylation of isoniazid and other drugs and glucose-6-phosphate dehydrogenase deficiency). Abundantly reviewed in the past (Kalow 1962; Motulsky 1964; Vesell 1973; Vesell and Penno 1983; LaDu 1972; Weinshilboum and Vesell 1984), these pharmacogenetic conditions will not be described in detail here. Each exemplifies how genetic constitution can determine drug toxicity. In general, toxicity arises in a genetically susceptible individual from accumulation of parent drug due to a block in its biotransformation to inactive metabolites. Transmitted in classical mendelian fashion by alleles at a single but different locus, each of these metabolic blocks is described as monogenic. In most cases, a double dose of a mutant gene is required to produce the defect (recessive phenotype), but rarely, as in phenytoin toxicity, does a single gene seem to suffice (dominant phenotype).

In 1968, the influence of genetic factors on drug response and toxicity was extended well beyond the aforementioned monogenic conditions to other entities encompassing different drugs (Penno et al. 1981; Vesell and Page 1968a, 1968b, 1968c). These new pharmacogenetic conditions involved drugs characterized by: (i) extensive hepatic metabolism through cytochrome-P-450-dependent monooxygenases and (ii) large interindividual variations in rates of elimination. Almost a dozen different drugs were given to normal

uninduced twins who were nonsmokers, otherwise unmedicated, and not chronic consumers of ethanol. These drugs included antipyrine (Penno et al. 1981; Vesell and Page 1968a), phenylbutazone (Vesell and Page 1968b), bishydroxycoumarin (Vesell and Page 1968c), ethanol (Vesell et al. 1971), halothane (Cascorbi et al. 1971), nortriptyline (Alexanderson et al. 1969), amobarbital (Endrenyi et al. 1976), phenytoin (Andreasen et al. 1973), salicylate (Furst et al. 1977), and tolbutamide (Scott and Poffenbarger 1979).

Genetic control over extensive interindividual variations in rates of elimination of these drugs was indicated by larger pharmacokinetic differences within dizygotic than within monozygotic twins (Penno et al. 1981; Vesell and Page 1968a, 1968b, 1968c; Vesell et al. 1971; Cascorbi et al. 1971; Alexanderson et al. 1969; Endrenyi et al. 1976; Andreasen et al. 1973; Furst et al. 1977; Scott and Poffenbarger 1979). Intraindividual variations were small compared to interindividual variations in these carefully selected, uninduced twins living under uniform environmental conditions. Although such observations supported a genetic basis for the large interindividual variations in the disposition of these drugs, the precise mendelian mode of transmission of these factors could not be elucidated just from the twin studies on parent drugs. Thus, when pedigree analysis was attempted solely on the basis of kinetic studies performed on the parent drug, rather than on principal metabolites, the mode of transmission of interindividual variations for bishydroxycoumarin (Motulsky 1964), phenylbutazone (Whittaker and Price Evans 1970), and nortriptyline (Asberg et al. 1971) was either unclear or else more compatible with a polygenic than a monogenic mechanism. In light of the current information discussed below, a polygenic attribution is misleading because more recent examinations of individual metabolic pathways have disclosed monogenic transmission.

Since some twins participated in several of these studies, it was possible to determine whether their rates of biotransformation of different drugs were closely correlated. Although some correlations occurred, rates of clearance of most drugs investigated were independent of one another (Vesell and Page 1968c). This result was notable for several reasons. It suggested that the twin studies had disclosed several discrete genetic entities, rather than one or two that determined large variations in the metabolism of numerous drugs. Furthermore, the existence of separate genetic entities, each involving a different molecular form (isozyme) of hepatic cytochrome P-450, agreed with simultaneously emerging evidence for extensive heterogeneity of this hepatic hemoprotein (Alvares et al. 1969; Lu and Levin 1974; Nebert et al. 1982; Ryan et al. 1975; Wang et al. 1980).

THE DEBRISOQUINE POLYMORPHISM

In 1977, a landmark report disclosed that approximately 7% to 9% of the population in the United Kingdom were genetically slow metabolizers of the antihypertensive drug debrisoquine (Mahgoub et al. 1977). After a 10-mg oral dose of debrisoquine, the phenotypes of extensive metabolizers (EMs) and poor metabolizers (PMs) were

distinguished in an 8-hour urine specimen by the ratio between the percent of the dose eliminated as the parent drug and the percent removed as its principal metabolite, 4-hydroxydebrisoquine. A ratio of 10 or more characterized deficient (poor) metabolizers of debrisoquine. This phenotype was conferred by a double dose of an autosomal gene. In patients treated with debrisoquine, toxicity occurred predominantly among deficient metabolizers. Thus, two alleles at a single genetic locus largely determine susceptibility to adverse effects from this drug.

Using sparteine rather than debrisoquine, Eichelbaum's group in Bonn reported a monogenically controlled variation which now appears to be identical in many respects to the debrisoquine polymorphism (Eichelbaum et al. 1979). In German subjects, the frequency of this inability to metabolize sparteine approximated that for debrisoquine in the United Kingdom. This meant that in the United Kingdom and Germany, for every 100,000 patients receiving usual doses of debrisoquine or sparteine, approximately 7,000 to 9,000 would be at risk of toxicity due to drug accumulation.

The group from the United Kingdom extended the scope of their discovery by suggesting a panel drug approach (Idle et al. 1979): slow metabolizers of debrisoquine were shown to be incapable of biotransforming several other drugs as well. Thus, deficient (poor) metabolism of debrisoquine was demonstrated to be associated with poor metabolism of nortriptyline (Bertilsson et al. 1980; Mellstrom et al. 1981); phenacetin (Sloan et al. 1978); phenformin (Shah et al. 1980); certain β-adrenoceptor blocking drugs such as metoprolol, alprenolol, bufuralol, timolol (Alvan et al. 1982), and propranolol (Shah et al. 1982a); guanoxan (Sloan et al. 1978); perhexiline (Shah et al. 1982b); encainide (Wang et al. 1984); and possibly D-penicillamine (Panayi et al. 1983). The genetic locus controlling debrisoquine metabolism could be designated "a gene of multiple effect." Since genetically deficient metabolizers of debrisoquine are also at increased risk of toxicity from decreased biotransformation of approximately 21 drugs, a clinically useful predictive test based on drug-oxidizing capacity seems to be indicated. In such genetically predisposed subjects and certain of their sibs, doses of these drugs could then be appropriately reduced or alternative drugs selected that are metabolized by other pathways of hepatic drug oxidation, thereby avoiding the dose-dependent adverse reactions that occur most frequently in the deficient phenotype. The metabolic conversion and pharmacological actions of numerous drugs are affected to varying extents and in differing ways by the debrisoquine polymorphism. Thus, the precise clinical consequences of the PM phenotype relate directly to the special properties of the particular drug administered (Idle et al. 1983):

(i) For drugs like the β-adrenergic blockers whose hepatic extraction is high and whose elimination is therefore blood-flow dependent, oral bioavailability would be enhanced perhaps fourfold or fivefold in PMs compared to EMs. The clinical consequences in the PM phenotype consist of exaggerated pharmacological responses to such drugs.

(ii) For drugs whose elimination requires metabolic oxidation, drug accumulation in PM phenotypes could produce toxicity, as in the neuropathy and liver damage after perhexilene that occur primarily in the PM phenotype (Shah et al. 1982b).

(iii) For the few drugs that require conversion to a metabolite before becoming pharmacologically active, such as encainide (Wang et al. 1984), where oxidative O-demethylation to desmethyl-encainide is necessary for antiarrhythmic effects, the PM phenotype will be resistant to therapeutic effects and, hence, deprived of benefit from the drug.

(iv) For drugs such as phenacetin, the PM phenotype may, due to blockage in one oxidative pathway, develop toxicity due to enhanced production of a toxic metabolite via a minor pathway. The PM phenotype is much more liable to toxicity than the EM phenotype because in the PM phenotype, when O-deethylation of phenacetin to acetaminophen is blocked, much more phenacetin than normal is subject to the aromatic oxidation and deacetylation to form hepatotoxic and hemolytic p-phenetidins (Sloan et al. 1978).

This list of consequences could be extended, but these examples suffice to illustrate the wide variety of clinical outcomes to be anticipated from polymorphic oxidation and how these clinical sequelae closely reflect the special metabolic profile and pharmacologic characteristics of the particular drug administered. Due to the considerable clinical consequences of polymorphic oxidation of several drugs, Idle et al. (1983) suggested that when administration of some of these commonly used drugs with low therapeutic indices is contemplated, patients should first be phenotyped to permit efficient and safe tailoring of dose to their individual metabolic requirements. To perform this service, regional centers were advocated (Idle et al. 1983). Furthermore, in new drug development where preclinical studies revealed metabolic oxidation as a significant part of the total elimination process, this fact could contraindicate further development of the drug or at least its use in PM phenotypes (Idle et al. 1983). While this suggestion may seem stringent, it could represent a theoretical possibility for a new drug that produces very high toxicity or lethality in the PM phenotype. Perhaps most such potentially dangerous new drugs never reach clinical trial, obviating the need for phenotyping. Jack et al. (1983) term "unwise" the suggestion that polymorphic or other types of variable hepatic metabolism of a new drug contraindicate its use or further development. They cite, to defend their position, wide benefit from propranolol, isoniazid, phenytoin, and many tricyclic antidepressants, none of which, they state, would have been marketed under such a restrictive policy. Finally, they consider it unnecessary to establish regional centers to phenotype patients before they receive polymorphically oxidized drugs. They believe that most physicians can safely individualize dosage of such drugs without prior phenotyping.

In addition to its numerous clinical applications, the debrisoquine polymorphism underscores several fundamental principles of pharmacogenetic investigation. For example, the debrisoquine polymorphism could only have been discovered after development of sensitive high-performance liquid chromatography techniques to detect and quantify the principal metabolite, 4-hydroxydebrisoquine. In pharmacogenetic studies, rates of formation of the main metabolites should be measured, not just elimination rates of parent drugs, particularly for drugs biotransformed by multiple pathways, because identification of principal metabolites permits phenotyping of subjects closer to their primary gene products (Kalow et al. 1977). Another technical aspect of the debrisoquine story is facilitation of genetic analysis by availability of a convenient, noninvasive method, the metabolic ratio, to phenotype subjects. Use of the metabolic ratio permitted rapid comparison of gene frequencies in different populations.

These population data, interesting from several points of view, demonstrate qualitative as well as quantitative variations in the gene in different groups and countries (Kalow 1984). Not only do allele frequencies change, but also genetic heterogeneity occurs; that is, presence of additional alleles at this locus in subjects from Ghana (Woolhouse et al. 1979, 1983). Comparisons of debrisoquine phenotypes in five different populations (Canadian Caucasian, Canadian Oriental, Saudi Arabian, Ghanian, and Nigerian) suggested greater complexity than initially thought: all five populations differed from one another in certain respects (see table 1 in Kalow 1984).

Genetic heterogeneity traditionally emerges after meticulous investigation, in different countries or regions, of a locus that appears initially, on the basis of limited population sampling, to possess only two alleles. From the point of view of pharmacogenetics, greater complexity of the debrisoquine polymorphism than originally anticipated has several implications. First, the nature of the alleles that occur commonly for this polymorphism should be characterized in a population and country before new drugs affected by this polymorphism are widely given, at least on only a single dosage schedule. Second, even though a screening procedure to define phenotypes may be satisfactory in a particular population, it may be unsatisfactory in another population where different alleles predominate. Specifically, Kalow considers the approach of phenotyping subjects according to their metabolic ratio of debrisoquine to 4-hydroxydebrisoquine in a single 8-hour collection of urine satisfactory in Caucasians but unreliable in native Africans (Kalow 1984; Inaba et al. 1981). Careful kinetic studies on the disposition of debrisoquine and its principal metabolite have been performed in Caucasian PMs and EMs to test the validity of the metabolic ratio as a method to phenotype subjects (Sloan et al. 1983).

Another contribution of the debrisoquine polymorphism has been stimulation of the search for other genetically controlled variations of drug oxidation. Extensive heterogeneity of the cyto-

chrome P-450 system also favored the premise that additional polymorphisms of drug oxidation exist. The search has been rewarding. New polymorphisms, distinct from debrisoquine, have been described for mephenytoin (Kupfer et al. 1981) and antipyrine (Penno et al. 1981; Penno and Vesell 1983). The antipyrine polymorphism was uncovered by application of a two-stage method that should enable still other polymorphisms to be identified.

POLYMORPHIC METABOLISM OF ANTIPYRINE

Even before the discovery in 1983 of a polymorphism of antipyrine oxidation independent of that for debrisoquine (Penno and Vesell 1983), its existence was suggested by circumstantial evidence. In the first place, twin studies in 1968 and 1981 revealed genetic control over large variations not only of antipyrine elimination (Vesell and Page 1968a), but also of rates of formation of the three main antipyrine metabolites (Penno et al. 1981). Second, PM phenotypes for debrisoquine and sparteine biotransformed antipyrine normally, not at reduced rates (Bertilsson et al. 1980; Danhof et al. 1981). Third, studies on induction of antipyrine metabolism in rats suggested that each of its three principal metabolites arose from a different hepatic cytochrome P-450 isozyme (Danhof et al. 1979; Inaba et al. 1980).

Analysis of 13 families provided evidence for a commonly occurring polymorphism of antipyrine metabolism maintained by two codominant alleles at one or more loci with frequencies of 0.8 to 0.9 and 0.2 to 0.1 (Penno and Vesell 1983). A common genetic mechanism regulating all cytochrome P-450 isozymes responsible for the three main antipyrine metabolites was suggested not only by the similar frequencies for the alleles that control variations in rates of formation of each antipyrine metabolite, but also by the significant correlation among these different rate constants within each subject (Penno and Vesell 1983). These observations are also consistent with a more complex interpretation that genetic control is maintained by some regulatory factors that are identical for all three loci and other such factors that differ at these loci.

Since the method developed to identify the antipyrine polymorphisms (Penno and Vesell 1983) permits searches for other polymorphisms and has just been used in our laboratory to define a new polymorphism of theophylline metabolism, it merits some discussion. The first step is to measure rate constants of formation of principal metabolites of a drug or of their partial clearances in a sufficient number of unrelated subjects (approximately 100) to generate distribution curves. Without this essential first step, pedigree analysis becomes uncertain due to the absence of an alternative way that is both quantitative and objective to determine the phenotype of each family member. Selection of 100 subjects follows from principles governing population genetics. Enough unrelated subjects need to be included in the sample so that a mode represents the rarer phenotype, either in homozygous or heterozygous form, i.e., the phenotype controlled by an allele q with a gene frequency of approximately 0.1 ($p = 0.9$; $q = 0.1$). If q were much less than 0.1, phenotypes

corresponding to q^2 (homozygotes) and 2 pq (heterozygotes) would be undetectable since this method is not feasible for screening the many hundreds of subjects that would be required. For example, if q were 0.01, only 1 in 1,000 subjects would be homozygous at this locus and only 2 in 100 would be heterozygotes. Some samples of 100 subjects might not contain even one heterozygote. Approximately 1 subject in 100 would be homozygous for an allele q if $q = 0.1$ (0.1^2); but even in the absence of that single subject from several samples of 100 subjects, the polymorphism would still be readily detected because 18 of the 100 subjects investigated (2 pq or 2 x 0.9 x 0.1) would theoretically be heterozygotes and phenotypically distinguishable on distribution curves and probits from the 81 subjects homozygous (0.9^2) for the more frequently occurring allele p.

Once constructed on the basis of results in 100 unrelated subjects, these distribution curves and probits serve to phenotype members of different families, possibly drawn from index cases at the far ends of the curve. Transmission patterns of these phenotypes which can be objectively assigned to each family member are then traced to determine whether they conform to a mendelian mode of inheritance. If so, this constitutes evidence for, but by no means unequivocally establishes, a genetic polymorphism of drug oxidation. If patterns of transmission in families are incompatible with mendelian laws, a monogenic mechanism is rejected. However, before a monogenic hypothesis is completely rejected, the family should be rechecked to assure that the drug-metabolizing capacity of each member was truly uninduced or uninhibited. For example, in the course of studies on the antipyrine polymorphism, we encountered only one family that contradicted a monogenic hypothesis (see figure 2 in Vesell 1984). On questioning, the mother admitted taking cimetidine; on restudy, after 3 weeks off cimetidine, she changed her values considerably, thereby making the transmission pattern in the family consistent with mendelian expectations.

When assessing the potential clinical significance of a genetic polymorphism controlling cytochrome P-450 isozymes subject to extensive environmental perturbations, one should recall several points. First, virtually all cytochrome P-450 isozymes are subject to environmental perturbation. Differences are mainly in the extent of induction. Furthermore, polymorphisms that may at first appear resistant may show, on further study, sensitivity to initially untested drugs, chemicals, or conditions. Second, additional alleles not encountered at a genetic locus on early study may appear later when different populations are tested. These new alleles might regulate cytochrome P-450 isozymes that are much different in inductive responsiveness. For these reasons, certain genetic polymorphisms of drug metabolism may possess more clinical significance and complexity than initially appeared, and the search for more such polymorphisms should be encouraged.

THERAPEUTIC AND TOXIC DRUG RESPONSES CONTROLLED BY GENES AT HLA LOCI

For reasons as yet unclear, some uncommon alleles at the HLA loci may be much more strongly associated with certain drug responses than

alternative alleles. For example, in schizophrenic patients, HLA-A1 has been reported to be highly associated with a favorable response to chlorpromazine (Smeraldi et al. 1976; Smeraldi and Scorza-Smeraldi 1976). By contrast, HLA-A2 appeared to be associated with an unfavorable response. In patients with affective disorders treated with lithium, HLA-A3 was associated with a high rate of relapse (Perris et al. 1979).

With respect to adverse reactions to drugs, in patients with rheumatoid arthritis treated with sodium aurothiomalate, adverse reactions (particularly proteinuria) occurred 32 times more frequently in the HLA-DR3 haplotype (Wooley et al. 1980). Also, in patients with rheumatoid arthritis, agranulocytosis after levamisole was encountered much more frequently in subjects with the rare haplotype HLA-B27 (Schmidt and Mueller-Eckhardt 1977). Hydralazine-induced systemic lupus erythematosis occurred two to three times more frequently in hypertensive patients with HLA-DR4 haplotype who received the drug (Batchelor et al. 1980). Another genetically determined phenotype, slow acetylation of hydralazine, also is associated with increased susceptibility to lupus (Perry et al. 1970). Although the acetylation polymorphism itself does not appear to be associated with any HLA haplotype (Batchelor et al. 1980), potential associations between other HLA haplotypes and different pharmacogenetic polymorphisms remain to be established and merit study.

OTHER PHARMACOGENETIC AREAS OF INVESTIGATION

Intriguing pharmacogenetic observations that require further study include increased sensitivity of certain ethnic groups to ethanol (Wolff 1972), mydriatic agents (Chen and Poth 1929), and diphenhydramine (Spector et al. 1980). In cultured cells, amplification of specific DNA sequences correlates highly with resistance to certain cytotoxic drugs, such as adriamycin (Roninson et al. 1984); elaboration of the mechanisms for this gene-environment interaction requires elucidation of the nature of the proteins encoded by the amplified DNA. These and other examples indicate the wide variety of ways in which genetic constitution can influence drug response. The pharmacogenetic approaches described above could be applied with the modifications described at the outset of the chapter to elucidate large interindividual variations that occur in response to certain drugs of abuse. Probably similar principles pertain and results of such studies could be informative and therapeutically useful.

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Studies on an Animal Model of Alcoholism

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INTRODUCTION

Alcoholism is a disorder characterized by a persistent and progressive pattern of aberrant alcohol-seeking behavior which, over time, results in the loss of control over drinking and the development of tolerance and physical dependence. As discussed by Dr. Cloninger in this monograph, there is now strong evidence that genetic predisposition plays an important part in the development of alcoholism in many people. Accordingly, the identification of biological risk factors and their underlying mechanisms of influence has become a high priority area of alcohol-related biomedical research for the present and the future.

It is noteworthy that there already exists a considerable body of evidence both in humans and in experimental animals that a variety of behavioral, physiological, and biochemical responses to ethanol have genetic determinants. Alcohol drinking behavior or preference is one such response (McClearn and Rodgers 1959; Eriksson 1968; Li et al. 1979). Others include central nervous system (CNS) (McClearn and Kakihana 1973; Riley et al. 1977) and systemic sensitivity (Mizoi et al. 1979) to ethanol, alcohol metabolizing capacity (Kopun and Propping 1977; Thurman 1980), the acquisition of tolerance to ethanol (Grieve et al. 1979), and the development of physical dependence on ethanol (Goldstein 1973). One experimental approach to gaining an understanding of biological predisposition would be to study how these other responses to ethanol relate genetically and mechanistically to normal and abnormal drinking behaviors. The validity of this approach is supported by recent reports that individuals who have the alcohol-flush reaction, caused by an abnormal elevation of circulating acetaldehyde, are less prone to becoming alcoholic than those who do not (Harada et al. 1983; Yoshihara et al. 1983). Activity of the low K_m aldehyde dehydrogenase isozyme is absent in tissues of individuals with this inherited trait, rendering them intolerant to the consumption of even small to moderate amounts of ethanol.

Investigators are searching for biological markers and potential mechanisms of genetic influence in children of alcoholics, who are a high-risk population. Dr. Schuckit reports his research on phenotypic markers in sons of alcoholics elsewhere in this monograph. The study of the relationship of alcohol drinking behavior to other heritable responses to ethanol in experimental animals requires the ready availability of animals willing to self-administer large amounts of ethanol. This feature is considered by most investigators to be a key element of any animal model for alcoholism. It is well known that most experimental animals avoid drinking ethanol solutions that are 10% (v/v) or higher in concentration when food and water are concurrently available. However, some strains of outbred laboratory rats do show a wide range of individual variation in alcohol preference and lines of rats exhibiting high and low alcohol preference have been raised through bidirectional selection (Eriksson 1968; Li et al. 1979). These successful breeding efforts have served to substantiate the existence of genetic influence on alcohol-seeking behavior, as have also the finding of differences in alcohol preference among inbred mouse strains (McClearn and Rodgers 1959). One line of alcohol-preferring rats, the P line, has now been systematically characterized with respect to its drinking behavior. The data indicate that it satisfies virtually all the preceived requirements for an animal model of alcoholism. Comparison of the effects of ethanol on P rats and on NP rats, a line simultaneously selected for alcohol nonpreference (aversion), has revealed differences that should provide insights into the biological basis of aberrant alcohol-seeking behavior. This chapter reviews the work performed on these selectively bred lines.

DEVELOPMENT OF AND STUDIES ON THE P AND NP LINES OF RATS

The suggested essential requirements of an animal model of alcoholism are (Lester and Freed 1973):

1. Ethanol should be self-administered orally, in preference to water or other solutions having the same caloric value and palatibility.

2. The amount consumed should produce measurable blood alcohol concentrations (BACs).

3. Ethanol should be positively reinforcing, i.e., the animals should be willing to overcome obstacles or work to obtain the alcohol.

4. The positive reinforcing effect of ethanol should be accountable in large part, if not entirely, by the CNS pharmacological actions of ethanol or its metabolic products and not because of its caloric value, taste, or smell.

5. Consumption should lead ultimately to
   a) repeated episodes of intoxication,

b) tolerance, and
c) physical dependence.

The P and NP lines were developed by selective breeding for high and low alcohol preference from a foundation stock of Wistar rats (Li et al. 1981). Testing was performed with an unflavored 10% (v/v) solution of ethanol made continuously available in a Richter tube to individually housed animals. Water in an identical Richter tube as an alternate source of fluid and solid food were provided ad libitum. The amounts of 10% ethanol, water, and food consumed daily were measured for 3 weeks and those animals exhibiting the highest and lowest consumption scores (g ethanol/kg body weight/d), respectively, were mated to start the next generation of P and NP lines. After 20 generations, the consumption scores (g/kg/d; mean ± SD) were: P males, 5.5 ± 1.2; P females, 7.3 ± 1.7; NP males, 1.1 ± 0.6; NP females 1.0 ± 0.9. The P and NP animals in the current S26 generation display similar characteristics. The P rats on test consume between 20% and 30% of the total calories as ethanol. They substitute the ethanol calories for a part of the food calories and gain weight at the same rate as control animals not given the ethanol solution as a fluid choice.

The P rats drink about 70% of the ethanol in the dark, when they also eat most of the food. Drinking occurs in bursts at irregularly spaced intervals (Waller et al. 1982a). When blood is sampled during the dark cycle, e.g., at the 3rd and 11th hours, BACs ranging from 14 to 120 mg% have been obtained. Mean values are about 60 mg%. When BACs are measured 30 minutes after the completion of drinking episodes, as determined by a drinkometer, values of 52 to 124 mg% have been obtained. The mean is 86 mg%. Clearly, these animals are attaining systemic alcohol concentrations that are pharmacologically active, at least for humans. Studies have also been performed to determine what BACs coincide with cessation of drinking and what blood levels produced by intravenous infusion would lead to curtailment of oral intake (Waller et al. 1982b). It was found that 50 to 70 mg% did both. It appears, therefore, that the higher BACs attained with oral intake represent overshoot, caused by delayed gastrointestinal absorption.

The P rats will work in order to obtain ethanol through operant responding (Penn et al. 1978). Free-feeding male animals were trained to bar-press for sweetened milk in a dipper as reward and the milk was then replaced with 10% ethanol. With food and water constantly available, they bar-pressed for the ethanol both on a continuous reinforcement schedule and when the bar-press to reinforcement ratio was increased. In fact, the response to reinforcement ratio could be raised to 6 or 7 before water intake increased, yielding a response rate of over 1,000 bar-presses/24 hours in each of the animals tested. Since food and water were freely available to the animals, it appears unlikely that the P animals found the ethanol solutions rewarding because of caloric needs or thirst.

The above studies, however, do not distinguish whether the reinforcing properties of ethanol are its pharmacological effects or its taste and smell. To dissociate the post-ingestive effects of ethanol from its orosensory cues, intragastric self-administration experiments were performed, using an experimental design similar to that reported by Deutsch and coworkers (Deutsch and Hardy 1976; Deutsch and Walton 1977). Male P and NP animals were surgically implanted with transesophageal catheters for the intragastric delivery of fluids. Following recovery, the animals were trained to associate: a) the intragastric delivery of ethanol with the drinking of a neutral-flavored (almond or banana) water solution contained in a U-shaped drinking tube; and b) the intragastric delivery of water with the drinking of the other neutral-flavored solution in another U-tube. The drinking of the fluids from the U-tubes activated a pump to deliver an equal volume of either the water or the ethanol solution intragastrically. After training, the animals were presented with both U-tubes and allowed free-choice drinking of the two flavored solutions on a 24-hour availability schedule. Food was freely available throughout the experiment. It was found that the P rats consistently self-infused greater volumes of the ethanol solution and lesser volumes of the water than did the NP rats (Waller et al. 1984a). This difference was observed regardless of whether the concentration of infused ethanol was 10%, 20%, 30%, or 40%. The amount of ethanol infused by the NP rats was always less then 1 g/kg/d at all concentrations of ethanol tested. By contrast, the amount of ethanol infused by the P rats increased from 3.0 $\pm$ 0.3 g/kg/d with 10% ethanol to 9.4 $\pm$ 1.7 g/kg/d with 40% ethanol. The BACs of animals measured 30 to 40 minutes after observed episodes of self-infusion of 20% ethanol were 116 to 303 mg% (mean of 199 mg%) and, with the self-administration of 40% ethanol, BACs were 92 to 415 mg% (mean of 231 mg%). All animals repeatedly showed signs of intoxication, such as ataxia and somnolence, after intragastric ethanol self-administration. These blood ethanol levels attained with intragastric self-administration were considerably higher than those observed in the P rats with free-choice drinking. Interestingly, although the total amount of 40% ethanol self-infused per day was significantly higher than the pretested voluntary oral consumption scores of the P rats (6.5 g/kg/d), the amount of 20% and 30% self-infused (5.8 and 7.3 g/kg/d, respectively) was not.

The above results are consistent with the notion that the post-ingestive, pharmacological actions of ethanol are rewarding to the P rats, but are aversive to the NP rats. However, the difference in BACs attained by the P rats with free-choice oral consumption and with intragastric self-administration suggests that orosensory cues also may be an important modulator of the amount of ethanol self-administered per drinking episode. It is important to note that Deutsch and coworkers (Deutsch and Eisner 1977; Deutsch and Walton 1977) were able to demonstrate intragastric self-administration of ethanol in large amounts in rats unselected for ethanol preference only if they were first made physically dependent by the prior forcible administration

of ethanol. Ethanol self-administration behavior was quickly extinguished in unselected animals not made ethanol-dependent, as was observed also in our study with the NP rats. In contrast, the P animals in this study were not made dependent on ethanol and, in fact, were ethanol-free for at least a month before these experiments. Clearly, the innate ethanol preference of experimental animals is an important if not crucial variable in studies of ethanol self-administration.

With chronic free-choice drinking of 10% ethanol, the P rats develop metabolic tolerance. After 6 weeks, the ethanol elimination rate of ethanol-consuming animals was 15% higher than that of control animals. Weight gain and total caloric intake of the animals in the two groups were identical. Ethanol represented 22% (week 1) to 30% (week 6) of the total calories consumed daily by the experimental group. The degree of metabolic tolerance developed over this period with free-choice drinking was the same as that developed by P rats forcibly given a total liquid diet in which 35% of the total caloric content was derived from ethanol (AIN liquid diet containing 5% ethanol). Studies are in progress to determine the time course and extent of neuronal tolerance development with free-choice drinking by the P rats.

We have also examined whether chronic free-choice drinking by the P rats produces physical dependence (Waller et al. 1982a). Experimental animals were given constant access to 10% ethanol and water for 20 weeks, while control animals received only water. Food was available ad libitum. After 20 weeks, the ethanol solution was taken away from the experimental animals and both groups were scored for signs of withdrawal by a blinded observer. In the first 24 hours following removal of ethanol, 18 of the 19 ethanol-exposed animals exhibited signs of withdrawal. These included Straub tail, broad based gait, tremulousness, hyperactivity, wet dog shakes, teeth chattering, induced running, and bizarre behavior. These manifestations abated after 72 hours. As expected, none of the control animals showed withdrawal signs. We concluded, therefore, that chronic free-choice drinking of 10% ethanol can produce physical dependence in the P rats.

The studies summarized above indicate that the P line of selectively bred rats should be a useful animal model for exploring the biology of alcohol-seeking behavior and conditions that promote and lessen this kind of behavior. To this end, we have performed a number of studies comparing the P and the NP lines. One way in which the lines differ is the degree of motor impairment produced by moderate to high doses of ethanol. In these studies, the animals were trained to jump onto a descending platform in order to avoid foot shock. The height to which the animals can jump was used as the measure of their motor performance. It was found that the NP rats took much longer to recover from the sedative-hypnotic effects of ethanol than did the P rats (Lumeng et al. 1982). This difference could not be

attributed to a difference in alcohol elimination rate between the lines nor to a difference in CNS sensitivity to ethanol. Rather, it was because the NP rats took longer to develop tolerance than did the P rats. This could be assessed by use of the jump test after the administration of a second dose of ethanol and the comparison of BACs at the time of recovery to criterion performance after the first and the second dose. It was found that, while both the P and NP rats developed acute tolerance, the P rats did so more quickly or to a greater degree than did the NP rats (Waller et al. 1983).

Although rapid tolerance development might be a mechanism that promotes and sustains high levels of consumption, it is unlikely to be a primary determinant of alcohol preference, since the BACs attained during free-choice drinking, usually less than 150 mg%, are substantially lower than those produced in the studies of tolerance development described above. Accordingly, a basis for the difference in drinking behavior between the P and NP rats was sought at lower doses of ethanol. It was found that the P rats exhibit increased spontaneous motor activity (SMA) following the intraperitoneal injection of ethanol, 0.065 to 0.5 g/kg. By contrast, the NP rats do not show stimulation, and decreased SMA began to occur with doses of 0.5 to 1.5 g/kg. The increase in SMA in the P rats is as much as 50% with 0.25 g/kg doses of ethanol (Waller et al. 1984b).

The combination of low-dose stimulation by ethanol and acute tolerance development to the high-dose effects offers an attractive hypothesis of alcohol abuse. This hypothesis assumes that the low-dose stimulation observed in the P rats is a reflection of the positively reinforcing or rewarding features of ethanol consumption, whereas the high-dose depressant effect is aversive and inhibits drinking. As tolerance to the high-dose effects of ethanol develops, the rewarding actions of ethanol become progressively extended into the higher dosage range, leading to increased consumption. We are currently testing this hypothesis in the P line of rats.

Studies have also been performed to discern whether there are neurochemical differences between the P and the NP rats. A major discovered difference is in the regional brain content of serotonin. Ethanol-naive P rats consistently have lower levels of serotonin in the cerebral cortex, corpus striatum, thalamus, hypothalamus, and hippocampus, and lower levels of 5-hydroxyindole acetic acid in the cerebral cortex and hippocampus, than do NP rats (Murphy et al. 1982). Although the significance of these neurochemical differences with respect to ethanol drinking preference is yet unclear, it is intriguing that serotonin and norepinephrine reuptake inhibitors are effective in reducing voluntary ethanol consumption in rats (Amit et al. 1984). These drugs display a similar action in the P rats, both on the 24-hour free-choice oral consumption schedule (Murphy et al. 1984) and with intragastric ethanol self-administration. Clearly, we are interested in the specificity of this kind of

response in relationship to the postulated roles of serotonin and/or norepinephrine in the reinforcing actions of ethanol.

SUMMARY

Past and ongoing studies indicate that the selectively bred P line of rats satisfies virtually all the suggested criteria for an animal model of alcoholism. They attain pharmacologically active levels of BAC and develop tolerance and physical dependence with voluntary oral ethanol ingestion, while in the free-feeding state. Ethanol is positively reinforcing to the P rats and consumption appears to be directed by the post-ingestive, pharmacological effects of ethanol, as revealed by the intragastric self-administration studies.

Some interesting differences between the P and the NP lines have been uncovered. They differ in the content of serotonin in several brain regions and they respond differently to ethanol. The P rats develop acute tolerance to sedative-hypnotic doses of ethanol more rapidly than do the NP rats, and they exhibit stimulation with low doses of ethanol. These differences suggest hypotheses on mechanisms underlying alcohol-seeking behavior which can now be tested experimentally.

It should be emphasized, however, that the described findings are the product of but a single genetic experiment. Clearly, replication is needed, and we are currently doing this, using a better defined, heterogeneous stock of rats. This one experiment, however, has demonstrated the feasibility of developing animal models of alcoholism and offers hope that the genetic and biological basis of alcohol-seeking behavior can be explored in the laboratory. The screening and testing of pharmacological agents able to deter alcohol-seeking behavior is an obvious practical application of this model.

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Development of DNA Probes to Investigate Genetic Variation of Alcohol Metabolizing Enzymes

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INTRODUCTION

Alcohol dehydrogenase (ADH) is responsible for the oxidation of a wide variety of primary, secondary, and aromatic alcohols, but is most noted as being the enzyme primarily responsible for oxidizing ethanol to acetaldehyde. Human ADH exists as a set of at least 15 different isoenzymes that differ in electrophoretic mobility, substrate affinities, and inhibition characteristics (Smith et al. 1973; Li 1977; Pares and Vallee 1981). All isoenzymes are similar in that they are dimeric, each monomer having a molecular weight of approximately 40,000 daltons. The various isoenzymes are categorized into three classes. Class I contains a large group of isoenzymes possessing various combinations of α-, β-, and γ-ADH subunits coded by three gene loci: ADH1, ADH2, and ADH3, respectively. Class II contains the π-ADH isoenzyme (Bosron et al. 1979; Li and Magnes 1975). Class III contains the χ-ADH isoenzyme, which oxidizes high molecular weight alcohols, but not ethanol (Pares and Vallee 1981). Class I and II are primarily liver-specific enzymes, although small amounts of activity of these isoenzymes occur in intestine, kidney, and lung (Smith et al. 1971). Class III ADH is a constitutive enzyme and is found in all cell types. Studies on the gene products of human class I ADH genes have revealed genetic polymorphisms.

Common genetic variation occurs at the ADH2 and the ADH3 gene loci. One of the variant forms of ADH2 is known to give rise to an altered ADH which differs in its kinetic properties and is implicated in differences in alcohol tolerance. The exact role of genetic variation in relation to propensity to develop alcoholism have been difficult to assess since this enzyme is primarily a liver-specific enzyme and is not readily analyzable in accessible tissues such as blood cells or serum.

We have initiated a research study aimed at isolating ADH and aldehyde dehydrogenase (ALDH) genes so that molecular probes may be derived to analyze genetic variation in the ADH and ALDH genes and their flanking regions. The results presented here relate to the derivation of DNA probes for the class I ADH genes.

Availability of partial amino acid sequence data for the β-ADH isozyme, coded by the ADH2 gene, enabled us to select a sequence of five amino acids with relatively low code degeneracy, near the carboxy terminal region of the ADH polypeptide. An oligonucleotide probe corresponding to this sequence was commercially obtained. The probe consisted of a mixture of 16 different oligomers, one of which is perfectly complementary to ADH mRNA (figure 1). The radiolabeled oligonucleotide probe was used to screen colonies derived from the adult human liver cDNA library of Orkin (Woods et al. 1982).

Ala	Asp	Phe	Met Ala	Amino acids 332-336
5'-GCN	GA^{U}_{C}	UU^{U}_{C}	Aug GCN-3'	mRNA sequence
3'-CGN'	CT^{A}_{G}	AA^{A}_{G}	TAC CG -5'	Synthetic oligonucleotide sequence

FIGURE 1

SEQUENCE OF OLIGONUCLEOTIDE PROBE (14 mer) FOR HUMAN ADH

One clone isolated from the cDNA library and designated pADH12 was found to contain an insert of 1100 base pairs. A restriction map of this clone was derived by further digesting the isolated cDNA insert by various enzymes. Restriction fragments were then cloned into the M13 bacteriophage and sequenced (Duester et al. 1984). Analysis of the DNA sequence of the insert in the pADH12 clone revealed that it contained a 593 base pair 3' untranslated region in addition to a 273 base pair translated region coding for 91 amino acids. The DNA sequence in this region predicted exactly the amino acid sequence of the terminal 91 amino acids in β-ADH as derived by Bühler and coworkers (1984).

This pADH12 cDNA clone has been used to derive genomic ADH clones. The Maniatis library of human DNA in lambda phage was screened with pADH12. Information from peptide mapping studies (Strydom and Vallee 1982) and partial sequence information on the ADH3 polypeptide γ (Bühler et al. 1984) indicate that the coding regions of the three class I ADH genes are closely homologous. Using radiolabeled pADH12 as a probe under conditions of low stringency, it should be possible to isolate from libraries of human genomic or cDNA, clones corresponding to all three class I ADH genes. One of the genomic clones, which was isolated from the Maniatis library using pADH12, has been found to contain a terminal exon, which is similar but not identical to that in pADH12 in addition to an adjacent intron and 3' untranslated region (Duester 1984). The base modifications present in the exon of the ADH53 clone correspond to those which one would expect to find in the ADH1 gene, based on the amino acid differences between β-and α-ADH polypeptide as determined by Jörnvall (personal communication, 1984)

INVESTIGATION OF ADH GENETIC VARIATION USING THE pADH12 AND THE ADH53 PROBES

Prior to using the ADH53 clone in studies of human DNA polymorphism, we ascertained that this clone did not contain any repetitive DNA sequences. Human DNA was isolated from peripheral blood leukocytes or from cultured hymphoblastoid cells. Human DNA was digested with a number of different restriction endonucleases, and individual DNA digests were subjected to agarose gel electrophoresis and Southern transfer to nitrocellulose filters (Southern 1972). Filters were hybridized with radiolabeled ADH gene probes and then washed at different stringencies prior to autoradiography. The restriction endonuclease MSP 1 has thus far proved most useful for detecting DNA polymorphism in the ADH genes (figure 2).

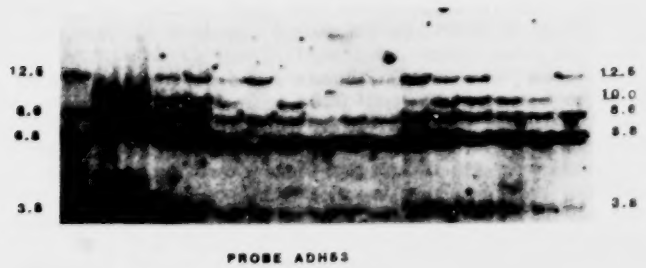


FIGURE 2

MSPI FRAGMENTS OF HUMAN GENOMIC DNA REVEALED BY HYBRIDIZATION TO ADH53 PROBE

Using the ADH53 probe and a low-stringency wash of the filters (1 x SSC, at 55 C for 40 minutes), four or five MSP 1 fragments are seen. These fragments are 12.5, 10.0, 8.6, 6.8, and 3.8 Kb in size (see figure 2 and figure 3). Occasional individuals have also been found to have a 4.2 Kb fragment. Following a high-stringency wash (0.1 x SSC/65 C/30 minutes) only the 10.0, the 8.6, and the 3.8 Kb fragments remain, suggesting that these fragments are most analogous to the ADH53 probe, which probably represents the ADH1 gene. Using the pADH12 probe, 4 fragments may be seen. These are the 12.5, 10.0, 8.6, and 6.8 Kb fragments. However, the 6.8 Kb fragment is frequently very weak with pADH12. Following a high-stringency wash, the 8.6 Kb fragment is the only fragment still visualized, suggesting that this fragment is most analogous to the ADH2 gene (see figure 3).

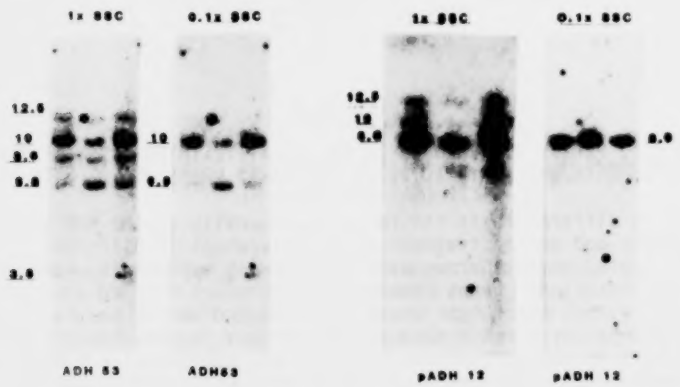


FIGURE 3

CHANGES IN PATTERNS OF DNA FRAGMENTS WHICH HYBRIDIZE TO ADH53 AND ADH12 UNDER CONDITIONS OF LOW AND HIGH STRINGENCY

In occasional individuals, an 11 Kb band may be visualized. This fragment is apparently more analogous to ADH2, based on the observation that it is usually more intense with pADH12 than with ADH53. The 12.5 Kb MSP 1 fragment hybridizes equally well with pADH12 and ADH53 at low stringency. At high stringency, it is not present with either probe. This finding suggests that the 12.5 Kb fragment may be derived from a third class I ADH gene, possibly the ADH3 gene which codes for the γ polypeptide. Individual variation has been observed with respect to the number of MSP 1 fragments. In some individuals, the 12.5 Kb fragment is absent, while in other individuals the 10.0 Kb fragment is absent (see figure 2). Individual variation has also been observed with respect to the relative intensity of the different fragments (see figure 4).

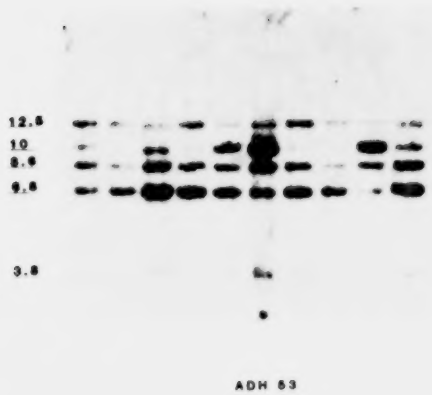
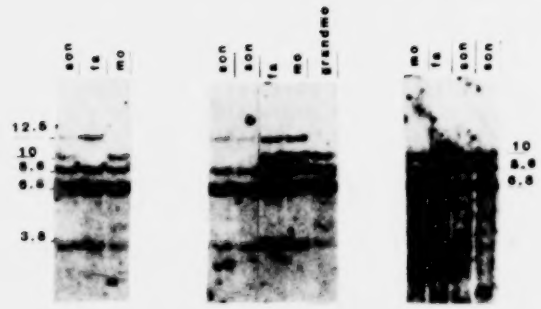


FIGURE 4

PERSON TO PERSON DIFFERENCES IN THE RELATIVE INTENSITIES OF THE DNA FRAGMENTS WHICH HYBRIDIZE TO THE ADH53 PROBE

Based on differences in the relative intensity of the MSP 1 fragments and on the fragment size differences in different individuals, we have drawn up the following hypothesis. One of the class I ADH genes (probably ADH3) gives rise to an MSP 1 fragment which hybridizes to ADH53 and pADH12 and is 12.5 Kb. A polymorphic site exists so that in some individuals a 10.0 Kb fragment results. However, the additional 2.5 Kb fragment which arises as a result of this polymorphism may not be visualized with ADH53 or pADH12. A second ADH gene (probably ADH1) gives rise to a 6.8 Kb fragment. In certain individuals anMSP 1 site is absent so that a 10.0 Kb fragment results. Individuals who are doubly heterozygous at the two loci described above will therefore exhibit a very intense 10.0 Kb fragment.

Examination of MSP 1 digestion patterns in a number of nuclear families has provided evidence of heritability of certain fragments. In figure 5a, the mother is homozygous for the 10.0 Kb fragment, while the father is homozygous for the 12.5 Kb fragment. Their son is an obligate heterozygote, having inherited the 12.5 Kb fragment from his father and the 10.0 Kb fragment from his mother.



ADH 53

FIGURE 5

ADH53 HYBRIDIZING MSP 1 FRAGMENTS PRESENT IN DNA FROM INDIVIDUALS IN 3 NUCLEAR FAMILIES

In figure 5c the father and the mother were both homozygous for the 10.0 Kb fragment. Their two sons were homozygous since each inherited the 12.5 Kb fragment from their mother and father. Note that in figures 5a and 5b there is also evidence of polymorphism with respect to the 3.8 and 4.2 Kb fragments. The exact relationship of this variation and the 12.5 to 10.0 Kb fragment variation is not clear at present. We have thus far examined DNA from 101 individuals, including 80 Caucasoids, 10 Orientals, and 11 Negroids. Polymorphism has been demonstrated in each population group.

In the analytical system described above, the presence of an MSP 1 fragment 10.0 Kb in size could result from polymorphism of either the ADH1 or the ADH3 gene. In an attempt to further resolve this polymorphism, we are currently carrying out double digestions of human DNA with EcoRI and MSP 1. In addition, studies are being carried out to derive gene-specific probes and/or gene-specific hybridization conditions. Further studies are also required to define the origin and significance of the 11 Kb band which occurs in occasional individuals and which is apparently more intense with the pADH12 (i.e., the ADH2) gene probe.

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Genetics as a Tool for Identifying Biological Markers of Drug Abuse

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A search for biological markers of drug abuse or alcoholism presupposes that some link can be made between the effects of behaviorally active drugs and some biochemical factor that is found in readily accessible tissues. Presumably, the identification of biological markers would be of value in that especially susceptible individuals could be diagnosed and warned of potential consequences of drug use.

The identification of a biological marker involves a search for some biological substance(s) which may be correlated with the biological substrates for drug dependence. Such a search requires, as a precondition, that drug dependence is due, at least in part, to biological factors. Since biological factors (enzymes, proteins, metabolic intermediates, anatomy) are regulated by genes, the search for a biological marker for drug abuse or alcoholism is essentially a search for gene products that are directly linked to or regulated by those genes that affect drug abuse related behavior. The search for such markers will be successful only if variation exists within the population with regard to drug abuse related behaviors. Presumably, variation will also exist within the population for some substance that is related to "drug abuse genes." Only if variation in susceptibility to alcoholism or drug abuse exists does it make sense to attempt to identify potential markers for these conditions.

The biological marker need not be influenced directly by those genes that influence drug abuse. An adequate marker could be the product of a gene that is closely linked to the drug abuse genes. Alternatively, pleiotropic effects of drug abuse genes may prove to be valuable markers. Pleiotropy is seen when a single gene influences more than one phenotype or trait.

Thus, the search for a biological marker may be a search for some gene product(s) that is directly related to drug abuse, a search for gene products that are regulated by pleiotropic effects of drug abuse genes, or a search for the products of genes that are closely linked to the drug abuse genes.

The evidence that supports the notion that genetic factors contribute to the development of alcoholism in humans is substantial and is reviewed in other chapters in this monograph. On the other hand, the evidence that indicates other forms of substance abuse are influenced by genetic factors is lacking--not because studies have been carried out that indicate that drug abuse is not influenced by hereditary factors but, rather, because in depth studies have not been carried out to assess this question. This suggests that a search for biological markers for alcoholism might be successful if initiated in the near future because appropriate populations may be more readily identified. On the other hand, a search for biological markers of drug abuse may be premature since the identification of adequate human populations for drug abuse may not be possible.

ANIMAL MODELS

A potential solution to the problem of lack of availability of suitable human populations for study is to develop animal models. This approach has been quite successful in alcohol research, and a number of genetically defined animal models related to alcoholism are now available. The progress related to the development of genetically defined animal models of drug abuse is considerably less, although enough data are currently available to indicate that successful models could be obtained. This chapter describes some of the progress that has been made in identifying potential models and also makes recommendations as to how animal models can be used to aid in the identification of biological markers of drug abuse.

The advantages of animal models in the search for biological markers of drug abuse and alcoholism are numerous. Perhaps the most important of these advantages relates to the potential need for invasive testing. Drug abuse and alcoholism are, in all likelihood, influenced to a major degree by central nervous system factors. Therefore, some factor(s) in brain are likely to be of primary importance in determining susceptibility to these afflictions. For example, it may be that the amount of drug receptor in specific brain regions is critical in determining whether an individual will be more likely to abuse the

drug which affects this receptor. Obviously, the availability of human brain tissue is not sufficient to test easily any hypothesis which requires measuring the brain content of some biological factor. It might take years, for example, to test the hypothesis that greater or lesser numbers of brain opiate receptors promote the abuse of opiates. Research involving humans would be totally impossible if the factor which regulates "addictability" is the degree of change in some neurochemical parameter, such as receptor number, during chronic drug use. Animal models can be of value in testing such hypotheses as well as in an efficient systematic search for gene products which are related to those genes that regulate changes in receptor number during chronic drug treatment.

If an animal model of drug abuse is to be developed, consideration must be given to what the model should represent. Should the model encompass every aspect of drug-related behavior or should it attempt to emulate only specific aspects? A complete animal model of human drug abuse might not be attainable. However, it should be possible to develop models of specific aspects of the total human condition. For example, it may be possible to identify or develop genetic stocks of animals that differ in avidity for specific drugs, initial sensitivity, tolerance development, and severity of a withdrawal syndrome. Once these models have been developed, they can be used to test hypotheses relevant to the mechanisms underlying the differences in avidity, sensitivity, etc., as well as being of value in the search for biological markers.

The prerequisite for identifying a biological marker for drug abuse is that some aspect of drug dependence is influenced by genetic factors. Therefore, it seems reasonable to suggest that the first step that should be taken is to ascertain whether genetically influenced variation exists in appropriate animal populations. In this regard, perhaps the easiest approach, because of the relative availability, is to screen inbred strains for differences in the phenotype (trait) of interest.

Inbred Strains

Inbreeding is achieved by mating closely related individuals. In commonly available rodent stocks, this has generally been achieved by brother-sister matings. Inbreeding leads to an increase in genetic uniformity within a strain, and after approximately 20 generations of inbreeding a strain can be considered genetically uniform; homozygosity will have been attained at all loci with the possible exception of those alleles which are carried on the sex chromosomes. The process

of inbreeding is nondirectional. Alleles are fixed in a particular configuration by chance with the exception of those traits that are related to reproductive fitness. Any differences found between inbred strains are fortuitous. In addition, no assurance can be made that a given inbred strain will have in its genome all of the genes that influence a drug-related behavior. Indeed, in view of the fact that most inbred rat and mouse strains can be traced to a small number of animals, in some cases two, it is highly probable that any strain will have only a sampling of all of the possible alleles that will affect a trait of interest.

A highly recommended approach regarding the use of inbred strains is to screen a number of strains. A relationship between two traits in a given strain may be unique. However, if this relationship is seen in a number of strains, it is more likely that these two traits are associated genetically. A major advantage of inbred strains is their constancy over time and in different laboratories. Such constancy increases experimental reliability and may allow collaborative studies that are carried out at different locations, thereby facilitating the screening of a large number of strains.

A classic example of consistent findings within inbred strains is the alcohol preference of various mouse strains; a number of investigators have noted that C57BL mice uniformly prefer ethanol-containing solutions, whereas DBA mice avoid ethanol-containing drinking fluids (McClearn and Rodgers 1959; Russell and Stern 1973; Randall and Lester 1975; Pickett and Collins 1975). Strain differences with respect to other alcohol-related behaviors have also been well documented. Included among these are strain differences in acute sensitivity to the effects of ethanol on locomotor activity (Randall et al. 1975; Oliverio and Eleftheriou 1976), passive avoidance conditioning (MacInnes and Uphouse 1973), body temperature (Moore and Kakihana 1978; Crabbe 1983), and duration of ethanol-induced loss of the righting response (sleep time) (Kakihana et al. 1966; Damjanovich and MacInnes 1973; Crabbe 1983). Strain differences in the development of tolerance to ethanol have also been described (Moore and Kakihana 1978), as have strain differences in the severity of an ethanol withdrawal syndrome (Kakihana 1979; Crabbe et al. 1983). The vast majority of the research relating to strain analyses has been descriptive in nature. Very few of these studies have attempted to determine such fundamental parameters as dominance relationships or an estimate of the number of genes involved in regulating the trait being measured; nor have the majority of

these studies attempted to provide biological explanations for these strain differences in ethanol-related behaviors. Clearly, very few of the studies concerning strain differences in alcohol-related behaviors have attempted to identify a marker that could be of value in predicting differential sensitivity to the trait being studied.

Strain differences in response to drugs of abuse have also been reported. For example, strain differences in response to amphetamines (Animan 1975; Animan and Kokkinidis 1975; Bovet and Oliverio 1967), nicotine (Morrison and Lee 1968; Hatchell and Collins 1977; Marks et al. 1983; Miner et al. 1984), hallucinogens (Lush 1975; Tilson et al. 1975), benzodiazepines (Rambert et al. 1976), barbiturates (Randall and Lester 1974; Belknap et al. 1973), and opiates (Castellano and Oliverio 1975; Horowitz 1976; Collins et al. 1977) have been reported. In general, these studies have involved examinations of different mouse or rat strains and have investigated strain differences in acute responses to these drugs. However, some of these studies have assessed strain differences in drug self administration (Horowitz 1976) or severity of a withdrawal syndrome (Belknap et al. 1973). As is the case with the research done in the alcohol field, few of these investigations have attempted to estimate gene number or dominance relationships and absolutely no concerted attempt has been made to identify potential biological markers. Nonetheless, the fact that strain differences have been identified argues that genetic factors are important in regulating response to drugs of abuse in animals. This finding supports the assumption that biological markers might be found.

Genetic Crosses

Once inbred strain differences in a drug-related response have been identified, genetic crosses can be developed. Such crosses can be invaluable in testing hypotheses of interest. For example, it seems highly likely that inbred strains which differ in some trait of interest could be identified. These strains will likely differ in a number of traits. Segregation analysis can be used to test the association between traits. Inbred strains could be crossed to obtain the first filial (F1) generation. If a number of strains are intermated in all possible ways, a diallel analysis can be made. Such an analysis allows an estimate of dominance relationships. Once the F1 generations have been obtained, these animals can be intermated to obtain the F2 generation. The F2 generation is invaluable for assessing linkage in that genes which are not closely linked will segregate in a quasi-independent fashion into the F2 generation.

Pickett and Collins (1975) used such an analysis to investigate the relationship between ethanol preference and brain serotonin content. Examples of other investigations that have used F1 analyses to investigate genetic regulation include studies of the effects of morphine on activity (Shuster et al. 1975; Castellano and Oliverio 1975) and the effects of amphetamine on activity (Bovet and Oliverio 1973). None of these studies have attempted to identify biological markers for the drug-induced behaviors being studied. However, the potential for such studies seems highly promising.

Recombinant Inbred Strains

Perhaps the most powerful tool currently available to test the relationship between two or more traits is recombinant inbred strains. Recombinant inbreds are constructed by first developing an F2 or preferably the F3 or F4 generations derived from crossing two inbred strains. Brother-sister mating is initiated in these foundation stocks. As was the case with inbred strains, genetic uniformity is achieved as inbreeding proceeds. The F3 or F4 generations are recommended as the foundation stock rather than the F2 because a maximum reshuffling of genes is obtained. Linkage is broken down and only genes that are very closely linked will be found in association with one another. This, of course, would be a very desirable trait for a biological marker. In theory, an F3 or F4 generation could serve as an adequate test group for a correlational analysis. However, since each animal is genetically unique,any variance involved in measuring the traits of interest will affect the correlation detected. For many traits, it may be desirable to have a test population where replicate samples can be obtained. Recombinant inbreds are ideal for such a situation because replicate samples can be obtained from each recombinant inbred stock. Any variance seen within each recombinant inbred provides an estimate of environmental influence on the traits of interest.

In view of the fact that recombinant inbreds are selected from F3 or F4 stocks derived from two inbred strains, these animals should be invaluable in testing linkage relationships. If two traits of interest are found in association in the parental inbreds and none of the recombinant inbreds demonstrate this association, it can be assumed that the traits were not closely linked in the originating inbred strains. If some, but not all, of the inbreds exhibit the traits of interest,it can be assumed that the genes which regulate these traits are closely linked. If 100% of the recombinant inbreds demonstrate both traits, identity of genetic control, pleiotropy, or extremely

close linkage probably explain the genetic relationship. Because knowledge of the precise genetic relationship between traits of interest would be invaluable when attempting to generalize any findings made in animals to the human condition, the use of recombinant inbreds in the identification of biological markers may be the most powerful tool currently available.

This reviewer could find no examples in the literature where recombinant inbreds have been used to study the actions of drugs of abuse. This is likely due to the fact that such animals are not readily available. However, Dr. Ben Taylor at Jackson Laboratories has developed a number of recombinant inbred mouse strains. The value of these animals in drug abuse related research could prove to be considerable, not just for the search for biological markers, but also in any experiment that attempts to assess the relationship between two or more behavioral, biochemical, or physiological traits.

Selectively Bred Lines

Selective breeding involves a systematic attempt to develop an animal with a specific trait. Unlike inbreeding, selective breeding is directional. Thus, the probability that two traits will be found in association with one another by chance in selectively bred stocks is diminished. Selective breeding will be successful only if the foundation stock displays genetically determined variation in the trait of interest. If this is the case, mating of extremes in the population (i.e., mating the highest with the highest and the lowest with the lowest) will result in a change in the mean value within the selected populations. If selection is accompanied by outbreeding, the problems associated with inbreeding are minimized; i.e., the high line(s) and the low line(s) will become similar genetically only at those genetic loci that affect the trait of interest. After approximately 20 generations of selective breeding, the high line will be homozygous at all of the genetic loci that contribute to a high value while the low line will be homozygous at all the loci that contribute to a low value of the trait of interest. Assuming that outbreeding has been maintained, the lines will exhibit the same diversity at all other genetic loci as existed in the founding population.

The founding population used to develop selected lines is extremely critical. The best population to use would be one that has maximum genetic diversity. Such a stock, usually referred to as a heterogeneous stock, can be developed by systematically breeding together

inbred strains. The best examples of such a stock are the HS mouse stock that is available at the University of Colorado Institute for Behavioral Genetics (McClearn 1972) and the National Institutes of Health heterogeneous stock rat strain (Hansen and Spuhler 1984). These stocks were derived by systematically breeding together eight inbred mouse or rat strains. Therefore, these stocks have considerable genetic diversity and the likelihood of identifying important genes or gene products is maximized. If a heterogeneous stock is not available, an F3 generation derived from two inbreds that differ maximally in the trait of interest may be suitable.

Selective breeding has been used successfully in a number of areas related to alcoholism. For example, a number of stocks have been developed that differ in alcohol preference (Mardones et al. 1950; Eriksson 1968; Li et al., this monograph). Most of these stocks were obtained by selectively breeding two lines that differ in free-choice ethanol consumption. Selectively bred lines of animals have also been obtained that differ in acute sensitivity to ethanol (McClearn and Kakihana 1981; Riley et al. 1977). One of these selections, that of McClearn and Kakihana (1981), involved the development of two lines of mice --Long-Sleep (LS) and Short-Sleep (SS)— that differ in duration of ethanol-induced anesthesia (sleep time). Riley et al. (1977) successfully selected two rat lines, Most Affected and Least Affected, that differ in the effect of ethanol on motor activity. Others (Goldstein and Kakihana 1975; McClearn et al. 1982) have developed mouse lines that differ in duration or intensity of a withdrawal syndrome following chronic ethanol treatment. Although these various lines have served as valuable tools in testing hypotheses that may explain the differing phenotypes, these animals have not been used in a systematic search for biological markers of alcoholism.

Unfortunately, very few investigators have used the selective breeding approach to study drugs of abuse. The only selection that this reviewer is aware of involving a drug of abuse involves morphine. Nichols and Hsiao (1967) selectively bred for high and low consumption of, or preference for, morphine after the rats had been chronically treated with morphine and withdrawn. This selection was quite successful in that a fourfold difference in consumption of morphine was detected as early as the third selected generation. Judson and Goldstein (1978) carried out an abbreviated selection study in Swiss Webster mice where lines that differed in levorphanol-induced alterations in motor activity were sought. After

three generations, a 3.5-fold difference between the high (runner) and low (nonrunner) lines was evident. More recently, Belknap et al. (1983) have reported their success in developing two lines of mice that differ in morphine-induced analgesia as measured with the hot-plate test. This selection is still underway. As of the fourth selected generation, complete divergence of the high and low analgesia lines has not been attained. Nonetheless, the high and low lines differ to a greater degree than do any of the inbred mouse strains that have been tested. Therefore, it seems reasonable to suggest that such animals would be invaluable in the identification of reasons for differences in sensitivity to morphine as well as in the identification of potential biological markers.

Selection processes also may be invaluable in identifying pleiotropic genes. An example that validates this assertion comes from the work of DeFries et al. (1978) who selectively bred lines of mice that differ in open field activity. These investigators selected duplicate high, low, and control activity mouse lines, i.e., six separate mouse lines. The foundation stock was an F3 generation derived from crossing BALB/cJ mice with C57BL/6J mice. Activity in an open field arena was measured. Two high lines, two low lines, and two control (randomly bred) lines were selected with outbreeding (avoiding common grandparents) being maintained. The replicate lines avoid or minimize potential problems associated with breeding of a finite number of animals. A steady divergence in activity has been obtained. Interestingly, within 10 to 15 generations, both of the low lines were nearly 100% albino. Apparently, the same gene(s) that influences coat color (albinism) also regulates locomotor activity in an open field arena. Thus, it could be argued that albinism in the mouse is a biological marker for a behavioral trait, i.e., low open field activity. DeFries has argued that low open field activity may well be an indication of high anxiety. If so, these mouse stocks could be valuable research tools for studying anxiety. Even if this is not the case, the DeFries activity mice provide considerable support for the notion that biological markers of drug abuse may be found if appropriate selectively bred lines are developed.

DISADVANTAGES OF ANIMAL MODELS

While animal models of drug abuse afford a number of advantages for the identification of biological markers, a number of potential disadvantages exist. Perhaps the most important of these is that commonly used laboratory animal stocks may not have the appropriate genome to develop a complete model that mimics

in all ways the human condition. It may not be accidental that alcoholism and drug abuse appear to be uniquely human conditions. Species other than man may not possess all of the genes required to elicit a full-blown dependence that resembles the human condition. Even where more limited aspects of drug dependence are being studied with animal models, caution must be taken when extending any potential findings in animals to humans. It is possible that animals differ in avidity for alcohol or drugs for reasons that are inoperative in humans.

Another potential disadvantage of developing animal models relates to the expense. If recombinant inbreds or selectively bred lines are to be developed, it will likely be several years before valuable animals will be generated. If selected lines are to be used, special consideration must be given to the selection phenotype. Unexpected results can sometimes be obtained. For example, we have recently determined that a very important factor which influences the difference between the LS and SS mice to an acute dose of ethanol relates to the fact that these animals were selected for differences in sleep time following the intraperitoneal injection of a 30% weight/volume ethanol solution (Gilliam and Collins 1983); i.e., some of the difference in response to ethanol between the LS and SS lines relates to the fact that the ethanol was administered via a route that is unimportant in man. Thus, before selection is even initiated, the response to be measured, the route of adminstration of the drug, and numerous other factors should be considered carefully. This is especially the case if the intent of the selection process is to emulate the human condition.

A number of additional cautions must be considered when a search for biological markers is undertaken. The fact that a given gene product seems to be linked to those genes involved in regulating drug abuse related behavior in mice or rats is no assurance that linkage will also be seen in man. If the gene product of drug abuse genes can be studied in both animals and man, the chances of correctly identifying a marker seem greater. The advantage of using animal models will be greatest if the direct gene products of drug abuse genes or pleiotropically regulated gene products can be identified.

CONCLUSIONS

The search for biological markers of drug abuse can be facilitated by the development of appropriate animal

models. These models offer the potential for invasive testing as well as the ability to more carefully control genotype than is the case in humans. However, disadvantages exist. These include expense and the possibilty that no animal model will completely satisfy the requirements needed to mimic the human condition. Nonetheless, the advantages far outweigh the disadvantages, particularly when the potential utility of animal models in answering important mechanistic questions regarding drug abuse is considered.

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Genetic Markers of Drug Abuse in Mouse Models

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The brain is a very complex organ. This complexity presents certain difficulties in the analysis of genetic determinants of responses to drugs of abuse. One gram of cortical grey matter contains about 200 million neurons. Each of these makes connections with several thousand other neurons. A single neuron can have receptors for several neurotransmitters. Neurons also exhibit plasticity--that is, the nature of their neurotransmitters and receptors can change with time. About 30,000 genes are expressed specifically by the brain (Sutcliffe and Milner 1984).

These characteristics permit several generalizations about the genetics of responses to neurotropic drugs. A drug response involves many separate components, such as drug metabolism; neurotransmitter turnover; peripheral as well as central responses; and interactions between several neurotransmitters, brain regions, and receptors. It is therefore reasonable to assume that a particular drug response, such as CNS stimulation, can be affected by many different genes. A corollary assumption is that most of the mutations that affect such a response will be pleiotropic. For example, a change in catecholamine metabolism that affects responsiveness to amphetamine could also alter spontaneous behavior, cardiovascular responses, etc. Furthermore, the same phenotypic response, such as increased locomotor activity after a stimulant drug, could result from mutations at several different loci.

In spite of these complexities, it is nevertheless possible to distinguish specific mutations that alter various responses to drugs of abuse. Work with mouse models provides some hope that we will eventually be able to identify and isolate genetic determinants of drug abuse and link them to known genetic markers.

In the following discussion, it is taken for granted that proof of a genetic component in drug responses is no longer necessary (Nebert and Felton 1976). The suitability of the various mouse models that are available will depend on the aims of any given research project. These aims can be classified as follows:

TO PROVIDE A REPRODUCIBLE MODEL OF GENETIC DIFFERENCES

Many inbred lines of mice are readily available from commercial suppliers. They were produced by over 20 generations of full-sib matings and are at least 99.9% homozygous. The simplest way to look for a strain difference in responses to morphine, cocaine, or ethanol is to consult Taylor's 1972 genetic similarity matrix. There is a wide divergence between C57Bl/6 and DBA/2, for example. Several differences have been documented in literature for the responses of these two strains to narcotics and to ethanol (table 1). Additional differences are described by Oliverio et al. (1983).

For example, in C57Bl/6 but not DBA/2 mice, morphine increases motor activity, the release of striatal dopamine, and the level of cyclic AMP in the striatum. DBA/2 mice, on the other hand, show more analgesia after morphine, sleep three times longer after ethanol than C57Bl/6 mice, and are susceptible to audiogenic seizures. Exposure to tobacco smoke or the injection of muscimol increases the motor activity of DBA/2 mice, but decreases the activity of C57Bl/6 mice. There are also striking differences in voluntary consumption. C57Bl/6 mice prefer dilute solutions of ethanol or morphine to water, but DBA/2 mice do not. Crabbe et al. (1981) reported that the pituitaries of C57Bl/6 mice contain twice as much beta endorphin as those of DBA/2 mice. Sprott (1975) has summarized behavioral differences.

Candidate strains can also be selected on the basis of differences in neurochemical parameters, such as dopamine turnover or norepinephrine content. These may be found in the compilation of Ingram and Corfman (1980). Mendelian analysis of differences in narcotic responses can be carried out by making appropriate crosses and backcrosses. Such crosses have demonstrated, for instance, that the stimulation of motor activity by morphine behaves as an autosomal dominant trait (Castellano and Oliverio 1975). Comparisons of C57Bl/6 and DBA/2 mice have also permitted some correlations between morphine responses and striatal dopamine activity (Racagni et al. 1979). In general, inbred lines provide readily available and consistently reproducible models of high responders and low responders. However, these lines differ in thousands of genetic loci and cannot easily be used to establish linkage or to isolate the determinants which control drug responses.

TO ESTABLISH LINKAGE

Bailey (1971) developed homozygous inbred lines from the F2 generation of crosses between two inbred strains. These recombinant-inbred (RI) lines provide a replicable sampling of progenitor genes in fixed combinations. Linkage markers are provided by histocompatibility testing of the RI lines and accessory congenic lines. The advantage of using RI lines is that extensive breeding is not required to establish linkage. However, they are most useful when the trait in question is under the control of a single

TABLE 1

Some Neuropharmacological Differences
Between C57BL/6 AND DBA/2 Mice

Drug or Treatment	Parameter	Response C57Bl/6	Response DBA/2	Reference
Morphine	Analgesia	↑	↑↑	Castellano & Oliverio 1975
	Motor activity	↑	-	Castellano & Oliverio 1975
	Striatal adenylate cyclase	↑	-	Racagni et al. 1979
	Striatal GMP	-	↑	Racagni et al. 1979
	Drinking preference	+	-	Horowitz et al. 1977
	Plasma cAMP & cGMP	↑	-	Muraki et al. 1982
	Caudate choline acetylase	↓	-	Ebel et al. 1980
Ethyl keto-cyclazocine	Analgesia	↑	↑↑	Gwynn and Domino 1984
Butorphanol	Analgesia	-	+	Filibeck et al. 1981
Naloxone	Motor activity	↑	↓	Castellano and Puglisi-Allegra 1982
	Discrimination learning	↓	↑	Castellano 1981a
	Withdrawal jumping after morphine	↑↑	↑	Brase et al. 1977; Gwynn and Domino 1984
FK 33-824	Motor activity	↑	↓	Castellano 1981b
Pentobarbital	Sleep time	↑	↑↑	Nabeshima and Ho 1980
Chloroform	Toxicity	resistant	sensitive	Hill et al. 1975
Ethanol	Motor activity	↓	↑	Kiianmaa and Tabakoff 1983
	Drinking preference	+	-	Rodgers 1966
	Sleep time after 3.3 g/kg	28 min	83 min	Spuhler et al. 1982
	Dose to inhibit neuronal firing	524	98	Spuhler et al. 1982
	Release of dopamine from striatum	↑	↑↑	Kiianmaa and Tabakoff 1983
Muscimol	Motor activity	↓	↑	Van Abeelen & Boersma 1984
Nicotine	Rate of respiration	-	↓	Marks et al. 1983
Tobacco smoke	Motor activity	↓	↑	Baer et al. 1980

(Table 1 continued next page)

TABLE 1 (continued)

Drug or Treatment	Parameter	Response C57Bl/6	Response DBA/2	Reference
Cycloheximide	Duration of inhibition of brain protein synthesis	7 hrs	3 hrs	Randt et al. 1971
Loud noise seizures	Audogenic	resistant	susceptible	Schlesinger & Sharpless 1975
Stress (defeat)	Analgesia	↑	↑↑	Shuster 1984
Electric Shock	Seizure	high	low	Schlesinger and Sharpless 1975

Responses are generally described as an increase (↑), a decrease (↓), or no change (-).

gene (Taylor 1978). Table 2 lists some RI analyses of responses to drugs of abuse. In most cases, the establishment of linkage has not been possible because multiple genes are involved.

One interesting benefit of testing RI lines has been the discovery of one line, CXBK, that is characterized by a low analgesic response to morphine and a decreased number of narcotic receptors in the brain (Baran et al. 1975). These mice also have a low analgesic response to acupuncture (Pomeranz 1978), to the injection of D-amino acids (Cheng and Pomeranz 1979), and to stress (Miczek et al. 1982).

Congenic lines can also be used to establish linkage. These are developed by repeated backcrossing to one progenitor strain from the F1 generation until homozygosity is achieved (Bailey 1975). The resulting lines have a very small portion of parental genome A, defined by histocompatibility testing, associated with the other parental genome B. If a congenic line which is over 99.9% congenic with parent B responds to a drug like parent A, then one can establish the linkage of that drug response to a defined portion of a particular A chromosome. Successful application of this approach to drugs of abuse has not yet been reported.

TO ISOLATE GENETIC DETERMINANTS

Selective Breeding

For careful genetic analysis of mechanisms of drug action, one would like to have two populations of animals which differ only in a single genetic determinant that controls a particular drug response. Selective breeding should, in theory, isolate those genes which control the selected drug response. The foundation stock should be genetically diverse. If necessary, such diversity can be achieved by interbreeding up to eight inbred lines, as was done in the selection of Long-Sleep (LS) and Short-Sleep (SS) mice for differential responses to ethanol (McClearn and Anderson 1979). The importance of carrying replicate lines and keeping stocks large enough to prevent inbreeding has been emphasized by Crabbe et al. (1983). They are developing lines that differ in the intensity of withdrawal after ethanol. Interesting correlations have been drawn between genetic susceptibility to the depressant effects of ethanol and the ability of ethanol to fluidize neuronal membranes (Goldstein et al. 1982). However, selective breeding is not without problems. Achieving homozygosity is slow and tedious. Until homozygosity is reached, it is almost impossible to replicate experiments, because the genotypes are continually changing. The drug tests upon which selection is based are likely to pick up pleiotropic genes which can affect the responses to a variety of CNS-active agents. For instance, the LS and SS lines also differ in their responses to barbiturates, adenosine derivatives, and other CNS depressants (Dudek et al. 1984; Howerton et al. 1983; Proctor and Dunwiddie 1984). Selective breeding for sensitivity to anesthesia by nitrous oxide has also revealed a lack of specificity (Koblin et al. 1984).

TABLE 2

The Use of Recombinant-Inbred Strains in Genetic Analysis of Some Drug Responses

Drug	Response	Conclusion	Reference
Chlorpromazine	Exploratory behavior	Two controlling genes, one on chromosome 9	Castellano et al. 1974
Scopolamine	Exploratory behavior	One locus on chromosome 4 controlling exploratory behavior, plus one on chromosome 17 determining response to scopolamine	Oliverio et al. 1973
Ethanol	Motor activity	Recessive allele Eam on chromosome 7 determines marked decline in activity	Oliverio and Eleftheriou 1976
Morphine analgesia	Hot plate	More than 2 loci	Oliverio et al. 1975
Morphine	Motor activity	More than 2 loci	Oliverio et al. 1975
Morphine	Tail-flick analgesia	2 or more loci no linkage	Shuster et al. 1975
Morphine	Motor activity	2 or more loci no linkage	Shuster et al. 1975
Morphine	Sensitization of motor response	no linkage	Shuster 1975
Naloxone	Binding to narcotic receptors	Two or more genes controlling number of receptors	Baran et al. 1975
	Withdrawal jumping	Multiple determinants	Shuster 1984
Opioids	Binding to brain membranes	Multiple genes	Reith et al. 1981; Jacob et al. 1983
Oxotremorine	Hypothermia gene	Single controlling	Lush and Andrews 1978
Cocaine	Liver damage	Multiple genes	Shuster 1984
d-Amphetamine	*In vivo* metabolism	Multiple genes	Shuster 1984

Defined Mutants

Hundreds of mutants of inbred mouse lines such as C57Bl/6 are available commercially and, in most cases, the locus involved has been established (Green 1981). Screening of these strains for their responses to drugs of abuse would be a fairly easy way to isolate genetic determinants. Of course, these genes would have to be pleiotropic because most of the mutants were first identified by a change in coat color or some other descriptive phenotype. Preliminary work by Katz and Doyle (1980) and by our laboratory (Shuster 1984) has turned up four mutants of C57Bl/6 that differ in one or more responses to morphine (table 3). It is intriguing that each of these mutations is found on a different chromosome.

There are other mutants, such as Jimpy, that involve defective development of the CNS (Law et al. 1978). These can differ in their responses to morphine, but the defects in the CNS are so widespread that these mice are not likely to be useful for studying mechanisms of action.

Sublines

Sublines of several inbred lines have developed as a result of genetic drift between isolated stocks. The number of gene differences can be calculated from the number of generations of inbreeding that took place before and after the stocks were separated (Bailey 1978). For example, the difference between the Jackson (J) and Bailey (By) sublines of C57Bl/6 is no more than 50 gene pairs. It is extremely improbable that more than one of these pairs affects the response to morphine. We have found that the By subline displays more analgesia after morphine than the J subline. Crosses and backcrosses have shown that this trait behaves as an autosomal dominant gene (Shuster 1984). Assay of narcotic receptors in the brain has revealed a greater number of mu receptors in the By mice, but no difference in delta or kappa receptors (Cremins and Shuster 1982). There are no obvious differences in the appearance of the two sublines. However, given the nature of genes controlling drug responses, it is not surprising that pleiotropic characteristics have been distinguished; e.g., By runs more than J after morphine (Shuster 1984). Also, there are differences in the climbing response to apomorphine and the running after amphetamine (table 4). Other sublines that have not been studied extensively should also provide useful material for investigating genetic determinants of drugs of abuse. For instance, the Jackson subline of BALB/c differs from the NIH, Bailey, and Texas sublines in that unrelated males of the J subline will fight viciously when housed together. There is a marked difference in the enzymes of catecholamine metabolism (Ciaranello et al. 1974) and in the sensitivity to d-amphetamine and ethanol (Moisset 1977, 1978). Here is another instance of a single pleiotropic gene affecting several drug responses.

TABLE 3

Responses of C57Bl/6J and Some Defined Mutants to Morphine

Mutant	Chromosome Locus	Response	Difference from C57Bl/6J	Reference
Pallid	2	Motor activity	↑	Katz and Doyle 1980
Pallid	2	Hypothermia	↑	Katz and Doyle 1980
Sepia	1	Analgesia	↑	Shuster 1984
		Motor activity	↑	Shuster 1984
Gunmetal	14	Analgesia	↑	Shuster 1984
		Motor activity	↑	Shuster 1984
Jimpy	X	Analgesia	↓	Law et al. 1978
Albino	7	Motor activity	↓	Katz 1980
Albino	7	Hypothermia	↓	Katz 1980

A greater response is indicated by ↑; a lesser response by ↓.

TABLE 4

Some Differences in Drug and Stress Responses Between the Bailey and Jackson Sublines of C57Bl/6 Mice

Drug or Treatment	Parameter	Response By	Response J	Reference
Morphine	Analgesia	↑↑	↑	Shuster 1982
Morphine	Motor activity	↑	↑↑	Shuster 1982
d-Amphetamine	Motor activity	↑	↑↑	Moisset 1977
Stress (defeat)	Analgesia	↑↑	↑	Shuster 1984
Dihydromorphine	Binding sites in brain	↑↑	↑	Cremins and Shuster 1982
Apomorphine	Climbing	↑↑	↑	Shuster, unpublished

By = Bailey ; J = Jackson

TO DERIVE NEW GENETIC MODELS

New methodologies in genetic engineering hold promise for more rapid development of new mouse strains that could be very useful for the study of drugs of abuse. For example, once parthenogenesis has been achieved as a reproducible technique in mice, both selective breeding and the development of new RI lines could be carried out much more rapidly because homozygosity could be achieved in only three or four generations (Csanyi 1984). Gynogenesis--that is, the production of diploid offspring from the fertilization of eggs with inactivated sperm cells followed by inhibition of the first mitotic division--has been carried out in fish, frogs, and newts (Csanyi 1984). Hoppe and Illmensee (1977) claimed to have produced gynogenic mice by cellular surgery, but their findings have apparently not been replicated in other laboratories (Anonymous 1984).

The pioneering work of Mintz (1978) provides an interesting new method for producing defined mouse mutants. Teratoma cells, cultured in vivo or in vitro, are integrated with embryonic cells of a different strain in order to produce a chimera. If the chimera's germ cells are derived from the teratoma, the chimera can give birth to mice bearing the teratoma genes. These mice do not develop tumors. Cultured teratoma cells can be exposed to mutagens and the mutants can then be selected. Mintz has used this technique to make a mouse model of hypoxanthine-guanine phosphorihosyltransferase deficiency (1978). Application of this technique to drugs of abuse raises the problem of which responses can be used in screening. Any tolerance or physical dependence produced in vitro is unlikely to be a function of genes that are specifically expressed in the CNS. However, it is conceivable that mutations affecting the structure and function of cellular membranes in general would be characterized by altered responses to ethanol and, perhaps, other drugs as well.

TO STUDY THE ROLE OF ENDOGENOUS OPIOIDS

There is considerable indirect evidence that the release of endogenous opioids plays an important role in many forms of stress-induced analgesia (SIA). We have found important strain and subline-differences in defeat-induced analgesia (Shuster 1984). Repeated defeat produces tolerance and cross-tolerance to morphine analgesia. Furthermore, tolerant animals exhibit withdrawal jumping when injected with naloxone, i.e., they are physically dependent (Shuster et al. 1983). A similar phenomenon has been reported (Christie and Chesher 1982) when mice are stressed by being forced to swim.

These observations suggest that suitable mouse models could be identified to test the hypothesis that repeated stress may produce a craving for drugs of abuse. They also indicate the potential value of testing drugs of abuse in strains of mice with different genetically determined patterns of behavior.

RECOMMENDATIONS FOR FUTURE WORK

It is clear from the foregoing survey that mouse models can provide very useful genetic tools for studying the actions of drugs of abuse. However, only a small fraction of the potentially useful material has even been surveyed for drug responses. It would be most helpful if NIDA could support the following initiatives:

1. Publication of a compilation of the known responses of inbred and recombinant-inbred strains, sublines, and mutants to drugs of abuse, including ethanol. This information is scattered through the literature. A compilation would make it much easier for researchers to choose appropriate models for testing hypotheses about drug abuse.

2. A systematic survey of drug responses in currently available strains and mutants which have not yet been tested. Perhaps such a survey could be carried out on a contract basis. Of the 500 or so known single gene mouse mutants, only about 15 have been examined even superficially.

3. Selective breeding for such parameters as voluntary consumption of ethanol and other drugs of abuse and ethanol. A good example is the selective breeding of strains of rats showing high and low preference for ethanol solutions (Li, this volume). A genetic model for self-administration of such stimulants as cocaine and amphetamine would be very helpful.

4. The development of new recombinant inbred lines to be used for genetic analysis. This development, like selective breeding, is a slow and tedious process that is not easy to support from short-term funding. If it could be carried out at NIDA, important new resources could be made available to the research community.

5. Increased emphasis on genetic correlations between behavioral parameters that can involve endogenous opioids and responses to drugs of abuse. These could include stress-induced analgesia, aggression, diurnal changes in activity and pain responses, etc. Also, the effect of stress and other pretreatments on voluntary consumption should be examined.

6. The selection of teratocarcinoma-derived mutants that differ in their susceptibility to drugs of abuse and/or neurochemical parameters, such as narcotic receptors, opioid peptides, and neurohormone turnover.

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Inheritance of Risk To Develop Alcoholism

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When alcoholism is assumed to be a discrete unitary trait, its inheritance is compatible with multifactorial inheritance (Cloninger et al. 1978). However, recent findings on ongoing studies in St. Louis and in Sweden of the inheritance of alcoholism indicate that alcoholism cannot be considered a homogeneous, discrete clinical disorder. Our studies indicate that the risk of alcoholism in the general population and in the relatives of alcoholics changes between generations, coincident with changes in social patterns of alcohol consumption. As patterns of exposure to alcohol change, the expression of underlying risk factors changes and the individuals at risk change. Our findings with adoptees have demonstrated the interaction of genetic and environmental factors in the development of alcoholism and have also distinguished multiple subtypes of alcoholism that have distinct genetic and environmental determinants.

Recognition of such gene-environment interaction and etiological heterogeneity will facilitate the identification of genetic and biological markers for alcoholism. Especially when social patterns of alcohol and drug intake change between generations, it becomes difficult to predict who is at risk and, more specifically, what is the risk of particular individuals. The implication is that our studies of biological and genetic risk factors in alcoholism and, more generally substance abuse, must take into account changing patterns of consumption and consider the interaction of biological and social risk factors in the development of clinical disorder. Thus, we must consider both familial and nonfamilial influences on biosocial risk factors.

SECULAR TRENDS IN ALCOHOL USE AND ABUSE

There are large group differences in the prevalence of alcoholism due to sociocultural influences. Both consumption and complications have varied widely from one historical era to another and currently vary from country to country, between social classes, between persons of different occupations, and between men and women (Cahalan 1970; Goodwin 1976). The significance of this variation is often ignored in biological and genetic research which presumes that alcoholism is a homogeneous disease entity

that is stably inherited from one generation to the next. Often it is asserted that about 25% of the first-degree relatives of alcoholics themselves will become alcoholic, with the implicit assumption or suggestion that this is not much influenced by secular or local differences in alcohol consumption.

Alcohol consumption per capita in the United States has increased nearly 50% in the past generation (25 years). Half of the alcohol drunk by Americans annually is consumed by about 10% of the population, and the risk of social and medical complications has increased along with overall consumption. This increased consumption and complication rate is reflected in findings about the risk of alcoholism in the general population and in the relatives of alcoholics. Recent results from our ongoing family study of alcoholics in St. Louis indicate that the risk of alcoholism in the general population and in the relatives of alcoholics is about twice the risk estimated 15 years earlier in St. Louis (table 1).

TABLE 1

Alcoholism in First-Degree Relatives of White Alcoholic Probands in St. Louis in 1968 and in 1983

Probands Sex/Year	Prevalence, % in General Population	Prevalence of Alcoholic Relatives	
		% of men	% of women
Men, 1968	11.4 (N=751)	36.0 (N=111)	6.7 (N=156)
Men, 1983	28.9 (N=1202)	62.7 (N=319)	20.6 (N=393)
Women, 1968	2.9 (N=608)	31.6 (N=38)	6.7 (N=60)
Women, 1983	4.3 (N=1802)	61.2 (N=67)	20.9 (N=91)

The estimates of the lifetime risk of alcoholism in 1968 were based on the survey of problem drinking described by Cahalan (1970). The estimates of the lifetime risk of alcoholism in 1983 is based on the recent report of the Epidemiological Catchment Area project concerning the St. Louis site by Robins et al. (1984). Alcoholism was diagnosed in relatives of alcoholic probands using criteria of Feighner et al. (1972) in a comparable fashion for both time periods. Both definite and probable alcoholics, using Feighner et al. criteria, are included so that

individuals with multiple social and medical problems are considered affected in table 1. It is difficult to prefer any particular diagnostic criteria over others because there is no work that demonstrates a discrete point of rarity or qualitative difference between alcoholic and nonalcoholic drinkers, such as that recently demonstrated for schizophrenia (Cloninger et al. 1985a) or somatoform disorders (Cloninger et al. 1984). In any case, the same diagnostic criteria were applied in comparable fashion in the two family studies (Winokur et al. 1970; Cloninger et al. 1978; Reich et al. 1980). The current pattern of sex differences is similar to that of 15 years ago: alcoholism is more common in men than in women, but the risk to relatives is the same regardless of the sex of the alcoholic proband. However, now the overall risk of alcoholism is about twice that observed less than a generation ago.

This difference between generations can also be demonstrated within our recent family study. This study includes personal interviews with the family members of 184 hospitalized alcoholics, 129 convicted felons with a history of alcohol abuse, and 47 non-alcoholic controls. This yielded 1,588 personally interviewed relatives. The risk of alcoholism in first-degree relatives of alcoholics, regardless of ascertainment source, decreases from the sibling generation (61% of brothers) to the parental generation (47%) even without correction for age (Reich et al. 1980).

GENETIC HETEROGENEITY AND GENE-ENVIRONMENT INTERACTION

Rather than assuming that alcoholism is a homogeneous discrete clinical disorder and asking whether or not it is heritable, we have sought to clarify the role of etiological heterogeneity, variable clinical expression, and the interaction of genetic and environmental risk factors in the Stockholm Adoption Study (Cloninger et al. 1981, 1984; Bohman et al. 1981, Bohman et al. 1984; Sigvardsson et al. 1984). The subjects included all 862 men and 913 women of known paternity who were born out of wedlock in Stockholm from 1930 to 1949 and adopted by nonrelatives at an early age. Most of the subjects were separated from their biological relatives in the first few months of life (average: 4 months), and all had their final placement in the adoptive home prior to age 3 years (average: 8 months). By Swedish practice at the time, neither the adoptee nor the adoptive parents were informed about the identity or behavior of the biological parents.

Extensive data about alcohol abuse, criminality, occupational status, and medical and social history were obtained for all adoptees and their biological and adoptive parents from several registers and medical sources. This includes the records of Temperance Boards and the National Health Insurance Board system, which includes local insurance agencies, hospitals, and clinics. The Temperance Board in each community evaluates reports of intemperance and then registers and imposes fines when intemperance is confirmed. It also supervises the treatment of alcoholics and may order involuntary hospitalization if needed. Other cases of alcoholism in adoptees were identified by records

of outpatient and inpatient care. The adoptees ranged in age from 23 to 43 years at the time of last information. The data were available for the lifetime of all subjects (adoptees and both sets of parents).

The risk of alcohol abuse in adopted men who had biological parents registered for alcohol abuse was compared to that in other adoptees (table 2). Alcohol abuse was significantly higher in the 268 adopted-out sons of biological fathers registered for any alcohol abuse than in the 571 sons of parents with no registered alcohol abuse (22.8% versus 14.7%, $\chi^2 = 8.27$, $P < .01$). In addition, of the 32 adopted-out sons of alcoholic biological mothers, the percentage of alcohol abusers was twice as high as for the 571 sons of nonalcoholic parents (28.1% versus 14.7%, $\chi^2 = 4.18$, $P < .05$).

The effect of parental alcoholism on adopted women was more complex (Bohman et al. 1981). The frequency of alcohol abuse was more than 3 times greater in the 51 adopted-away daughters of

TABLE 2

Inheritance of Susceptibility to Alcohol Abuse in the Stockholm Adoption Study

Alcohol Abuse in Biological Parents		Alcohol Abuse in Adoptees			
		Sons		Daughters	
Father	Mother	N	%	N	%
No	No	571	14.7	577	2.8
Yes	No	259	22.4	285	3.5
No	Yes	23	26.0	29	10.3
Yes	Yes	9	33.3	22	9.1

alcoholic mothers than in the 577 daughters of nonalcoholic parents (9.8% versus 2.8%, $\chi^2 = 7.17$, $P < .01$). In contrast, the excess of alcohol abusers among the 307 daughters of all alcoholic fathers was not significant (3.5% versus 2.8%). However, further analysis revealed that there was a twofold excess of alcoholic women among the 120 adopted-out daughters whose congenital background was characterized largely by biological fathers who had single registrations with the Temperance Board and no associated criminality (6.7% versus 3.0%, $\chi^2 = 4.51$, $P < .05$) (Bohman et al. 1981). Fathers with recurrent alcohol abuse or criminality had no excess of alcoholic daughters.

Social factors influenced the expression of risk for alcohol abuse and, in particular, the frequency of registrations with the

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Temperance Board. Biological fathers with single registrations for alcohol abuse did not increase the risk of their sons for either single or recurrent registrations (table 3). However, fathers with recurrent registrations had an excess of sons with either single registrations (11.6% versus 6.6%) or recurrent registrations (15.7% versus 8.6%). Thus, single registrations for abuse appear to be an expression of the heritable risk for alcohol abuse in the sons, but not in the fathers. This inconsistency is largely explained by differences in the social circumstances of the biological fathers and their adopted-away sons.

TABLE 3

Registrations for Alcohol Abuse in Biological Fathers and Their Adopted-Away Sons

Father's No. Registrations	Number Sons	Registrations in Adopted-Away Sons	
		% only one	% recurrent
none	594	6.6	8.6
only one	96	5.2	9.4
recurrent	172	11.6	15.7

The occupational status of the biological fathers tended to be lower than that of the adoptive fathers, and the occupational status of the adoptive father influenced the expression of risk for developing alcoholism. Table 4 shows the frequency of alcohol abuse registrations in the sons in relation to the number of registrations of the biological father and the occupational status of the adoptive father. Adoptive fathers with unskilled occupations had more sons with alcohol abuse than other adoptive fathers. If the adoptive father had a skilled occupation, there was an increased risk of alcohol abuse (single or recurrent) in sons of biological fathers with recurrent, but not single, registrations. In contrast, if the adoptive father had an unskilled occupation, there was an excess of recurrent alcohol abuse in sons of biological fathers with either single registrations (17.2% versus 7.6%, $\chi^2 = 3.29$, $P < .06$) or recurrent registrations (19.0% versus 7.6%, $\chi^2 = 8.52$, $P < .01$). Thus, severity of alcohol abuse is a complex product of biological and social determinants.

The only significant risk factor about the adoptive parents themselves was low occupational status of the adoptive father. Alcohol abuse in the adoptive parents was not associated with a greater risk of alcohol abuse in the adoptees. Only 13% of the 31

TABLE 4

Occupational Status of the Adoptive Father and Frequency of Registrations for Alcohol Abuse in Biological Fathers and Their Adopted-Away Sons

Adoptive Father's Job	Biological Father's Registered	Number of Sons	Registration of Sons	
			% once	% recurrent
skilled	none	406	6.2	7.6
skilled	once	67	4.5	6.0
skilled	recurrent	109	12.8**	13.8**
unskilled	none	188	7.4	10.6
unskilled	once	29	6.9	17.2*
unskilled	recurrent	63	9.5	19.0***

* P<.10 compared to 406 low-risk sons
** P<.05 compared to 406 low-risk sons
*** P<.01 compared to 406 low-risk sons

men reared by an alcoholic adoptive parent abused alcohol themselves compared to 18% of the other 831 sons. Only 1 (3.7%) of the 27 daughters reared by an alcoholic adoptive parent became an alcohol abuser compared to 3.4% of the other 886 daughters. Thus, imitation of parental alcohol abuse does not appear to be an important cause of the familial aggregation of alcoholics.

THREE DISTINCT GENETIC TYPES OF ALCOHOL ABUSERS

By a series of independent tests, we have been able to distinguisn three types of alcohol abusers with distinct clinical features and different genetic and environmental backgrounds (Bohman et al. 1984). These types have been called milieu-limited alcoholism, male-limited alcoholism, and antisocial behavior disorder with alcohol abuse. The clinical picture of men and women in each of the three types of families is summarized in table 5.

The validation of this typology has proceeded by three steps. The distinction between milieu-limited and male-limited alcoholism was first identified by subdividing the male adoptees according to severity, in accord with earlier clinical work by Kaij (1960). Among the biological mothers and fathers of mild abusers, there was an excess of mild abuse, but no criminality. Moderate abusers had an excess of recurrent alcohol abuse complicated by criminal behavior in their biological fathers, but no excess of alcohol

TABLE 5

Description of Three Family Types of Alcohol Abusers

Family Type	Men	Women
Milieu-Limited	Alcoholism usually mild adult-onset no criminality	Alcoholism usually mild adult-onset no criminality
Male-Limited	Alcoholism recurrent teenage-onset alcohol treatment crime due to abuse	Somatization low frequency diversiform
Antisocial	Criminality repeated violence untreated abuse	Somatization high frequency hypochondriacal

abuse or crime in their mothers. Severe abusers were heterogeneous: most had biological parent backgrounds indistinguishable from mild abusers, but had more deprived adoptive parent backgrounds than the mild abusers; other severe abusers had biological parents with prominent criminal backgrounds.

Environmental variables did not influence the adoptees' risk of moderate abuse, which was highly heritable from father to son. This male-limited group of alcoholic families was distinguished from others in which both fathers and mothers were at increased risk for alcoholism and the alcohol abuse was less severe and unassociated with criminality. The latter group was called milieu-limited because the sons were not at increased risk for alcoholism unless they had an adoptive father with low social status in addition to a biological parent with mild abuse.

The proposed distinction between these two types was first tested by predictions about the biological parents of alcoholic women. The biological parents of the 913 adopted women were classified according to the typology derived using discriminant analysis of the men (Cloninger et al. 1981; Bohman et al. 1981). We predicted that daughters in families of milieu-limited, but not male-limited, alcoholic parents would be at increased risk for alcoholism. This prediction was confirmed. This confirmation of different inheritance patterns in our single large population may reconcile the apparent discrepancies between earlier adoption studies which were too small to take such heterogeneity into account (Cloninger et al. 1985b).

The women in the families of male-limited alcoholics had no excess of alcohol abuse, so we evaluated their lifetime medical histories and records of sick leave disability which are maintained for nearly all Swedish citizens through their National Health Insurance Board. We found that 10% of Swedish women account for half of all sick leave disability, and that these "somatizers" are characterized by recurrent complaints of headache, backache, and vague abdominal symptoms from an early age (Sigvardsson et al. 1984). Further work revealed that these somatizers included women with two distinct clinical disorders that were each highly heritable but independent of one another: "high frequency" somatizers who have frequent disability from abdominal, back, and psychiatric complaints; and "diversiform" somatizers who have less frequent, but more diversely distributed, physical complaints and rare psychiatric complaints (Cloninger et al. 1984). Using this independently derived classification, we found an excess of women with diversiform somatization in families of male-limited, but not milieu-limited, alcoholics (Bohman et al. 1984). This strongly supported the distinction between the two types of alcoholics derived from the male sample, and indicated that the same genetic predisposition had different expressions depending on the sex of the individual.

High frequency somatization was not associated with either male-limited or milieu-limited alcoholism. However, the male relatives of these women were characterized by prominent violent criminal behavior; they often had multiple registrations for alcohol abuse, but this seldom required medical or psychiatric treatment (Bohman et al. 1984). These families appear similar in clinical features and mode of inheritance to families in which antisocial personality in men aggregates with Briquet's syndrome in women, using diagnostic criteria and subjects from the United States (Cloninger et al. 1975).

IMPLICATIONS FOR FUTURE RESEARCH

Alcoholism is a complex phenotype that appears to be influenced by multiple genetic and environmental determinants. In order to characterize its inheritance, we must take into account changing social patterns of consumption. Also, distinct familial subtypes with different patterns of gene-environment interaction need to be distinguished in order to obtain consistent replicable results. Our demonstration of genetic heterogeneity in the Stockholm Adoption Study makes it possible to reconcile the discrepant prior reports of Roe et al. (1945) and Goodwin et al. (1973). Roe's findings are consistent with those observed in our milieu-limited alcoholics, whereas Goodwin's findings are similar to our male-limited alcoholics.

Likewise, prior difficulty in obtaining consistently replicable results with biological and genetic markers in alcoholics may be attributed to the etiological heterogeneity we have observed. Individuals in families with alcoholic women more often have resting electroencephalograms (EEGs) with little alpha activity and poorly synchronized wave patterns than do individuals in

families in which only the men abuse alcohol (Propping et al. 1981). This pattern, called the borderline alpha EEG, is heritable and shows a dramatic increase in alpha activity in response to alcohol that is coincident with a pleasant feeling of calm alertness and tension relief, thereby reinforcing the tendency to drink alcohol in affected individuals (Propping et al. 1980). These findings are suggestive of an association with milieu-limited alcoholism, but not male-limited alcoholism. In contrast, preliminary work in Sweden has demonstrated an association of low platelet monoamine oxidase activity in male-limited alcoholism, but not milieu-limited alcoholism (von Knorring et al., in press). Inconsistent prior findings may be due to neglect of such clinical and etiological heterogeneity.

Further work is needed to specify and refine explicit diagnostic criteria for clinical subtypes of alcoholism and to identify the biological and genetic markers which differentiate these disorders. Recent advances in recombinant DNA technology (Cloninger et al. 1983) are leading to rapid increases in our knowledge of the human chromosome map. Quantitative methods for pedigree analysis (Reich et al. 1980), improved specification of clinical subtypes, and improved power to detect linkage when it is present should allow more rapid and consistent progress than in the past. High priority should be given to studies of families in which detailed clinical data can be related to putative biological and genetic markers.

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Genetic and Biological Markers in Alcoholism and Drug Abuse

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This chapter reviews a research approach that can be used in the search for genetic trait markers of a vulnerability toward substance abuse. Reflecting the pattern of studies in the literature, emphasis is placed on alcoholism, but the possible application of findings to other substances is discussed.

Study of a predisposition toward substance abuse is a complex undertaking. The human body has 70 trillion potential genotypes, only 5% to 20% of which are active at any one time, and there is a long lag between the potential onset of gene expression and the development of the final clinical picture which might be labeled substance misuse (Schuckit 1985a).
The comments offered here begin with discussions of data supporting the importance of genetic factors in alcoholism and other drugs of abuse. I then describe a specific model used in our laboratory for searching for phenotypic markers associated with a risk for alcoholism. Finally, I discuss the potential research and clinical implications of these findings and suggest some future directions for research on vulnerabilities toward misuse of substances other than alcohol.

GENETIC FACTORS IN ALCOHOLISM

Data generated in the last decade from family, twin, and adoption studies point to the importance of genetic factors in alcoholism (Schuckit 1984a). Virtually all family studies have shown the familial nature of alcoholism, while studies of identical twins have documented a higher concordance rate for alcoholism than in fraternal twins. Other twin studies point to genetic control of drinking behavior (Partanen 1966); alcohol metabolism (Vesell et al. 1971); and some alcohol consequences, such as cirrhosis and psychoses (Hrubec and Omenn 1981). Half-sibling and adoption studies document a fourfold increased risk for alcoholism in the adopted-out sons and daughters of alcoholics separated from their biological parents in infancy and raised without knowledge of their parents' drinking problem (Schuckit et al. 1972; Goodwin et al. 1974; Bohman et al. 1981;

Cloninger et al. 1981). However, being raised by an alcoholic parent figure, but with no alcoholic biological parent, does not seem to increase the risk for alcoholism.

Finally, animal studies have demonstrated a genetic influence on alcohol drinking behavior, central nervous system (CNS) sensitivity to alcohol, and the rate of development of tolerance (Meisch 1982). Dr. T.K. Li (this volume) has discussed these studies and the development of alcohol-preferring rats, a potentially powerful tool for alcohol research.

GENETIC STUDIES IN DRUG ABUSE

Unfortunately, information supporting the genetic influence in abuse of substances other than alcohol is scarce and available results are inconsistent. Several scientists have bred rat and mouse strains for morphine-seeking behavior (Nichols and Hsiao 1967; Ericksson and Kiianma 1971), and there is some evidence that there may be an overlap in an animal's preference for ethanol and morphine or phenobarbital (Sinclair et al. 1973). In animals, at least, one might therefore be dealing with a preference for substances that may cross over from ethanol to barbiturates or opiates. This hypothesized interaction is consistent with clinical observations that potentially lethal overdoses of ethanol might be reversed by naloxone (Dole et al. 1982).

Human genetic studies in drug abuse are also relatively rare. Family studies have revealed a trend for preference of similar types of drugs within families, and monozygotic twins show greater similarity for drug preference or response than dizygotic pairs, although not all researchers agree (Gershon 1980; Tennant 1976; Pederson 1981; Liston et al. 1981).

Although there have not been enough studies of the importance of genetic factors in substance abuse other than alcohol to draw even tentative conclusions, there are a number of tools that might be considered for application in the substance abuse area at the appropriate time. The next section addresses one model.

A MODEL FOR SEARCHING FOR PHENOTYPIC MARKERS IN ALCOHOLISM

There is now enough data supporting genetic influences in alcoholism to justify a search for the mechanism by which the increased vulnerability is expressed. One approach uses populations at elevated risk for the future development of alcoholism, concentrating on individuals before the actual abuse develops. These populations have been chosen because of demographic characteristics or because of the presence of close family members who abused substances (Schuckit 1985a).

Our laboratory is currently using a model for searching for phenotypic markers that might be associated with the vulnerability toward alcoholism. Our approach is based on the assumption

that alcoholism is probably a polygenic and multifactorial disorder. It is likely that a number of different types of genetic vulnerabilities interact with cultural and social factors, such as availability of alcohol (including the price of alcohol and distributing laws) and attitudes toward drinking and drunkenness. Once alcoholism develops, there may be a different set of factors that perpetuate it. As an example, I will discuss the results from our evaluation of whether the level of acute reaction to alcohol might contribute to the predisposition toward alcoholism.

Since sons of alcoholics have a significantly higher risk for this disorder than sons of nonalcoholics, these men are the focus of our work. Our sample was drawn from over 1,000 male university students and nonacademic staff, aged 21 to 25, who responded to a yearly mailed survey (Schuckit 1984b; 1985b). Individuals who were already abusing drugs or alcohol or who had psychiatric or medical problems were excluded from study. Of the remainder, sons of alcoholics (family history positive or FHP) who were themselves drinkers, but without substance-related major life problems, were identified. For every FHP man, a family history negative (FHN) control matched on age, race, religion, sex, educational level, drinking pattern, and drug-taking history was selected.

In Phase 2 of our study, FHP/FHN matched pairs were individually invited to the laboratory on three occasions where they drank a placebo, or 0.75 ml/kg of ethanol, or 1.1 ml/kg of ethanol (the equivalent of about three and six drinks, respectively) under standard conditions. Before testing, the drinking history was reevaluated by interview and through analysis of blood alcohol concentration (BAC) and a series of blood tests likely to change during heavy drinking (e.g., gamma glutamyl transferase, mean corpuscular volume, triglycerides, and uric acid) (Schuckit 1984c). In addition, the subjects' expectations of feelings after three drinks were measured (Schuckit 1984b). Figure 1 shows BAC over time for 34 FHP and 34 FHN men. The time to peak BAC and the rate of disappearance of ethanol in the two groups were virtually identical after both doses. FHPs and FHNs also indicated virtually identical expectations of how alcohol would make them feel (Schuckit 1984b).

The actual levels of drug effect and feeling of intoxication experienced after consuming the beverage during the test sessions was measured on a 36-point analog scale with 0 indicating no high feeling at all and 36 indicating the highest possible. As illustrated in figure 2, subjects receiving a placebo reported a small increase in perception of an ethanol effect at 60 minutes, while subjects receiving active ethanol doses reported marked changes in feelings (Schuckit 1984b). The FHN men, however, reported significantly more intense subjective ethanol effects than the FHP men after the low dose (0.75 ml/kg). Following the higher ethanol dose (1.1 ml/kg) (figure 3), the group differences, while present, were not as marked. Therefore, despite

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identical group BACs, similar expectations of the effects of ethanol, and similar drinking histories, FHNs expressed more feelings of drug effects, especially after the lower dose challenge.

We have also evaluated whether the family groups differed on more objective measures of the effects of alcohol. For example, each subject's level of standing steadiness or body sway was evaluated by asking them to stand with their feet together, eyes open, and hands at their side. Each person was studied at baseline during each of the three sessions, and then repeatedly every 30 minutes or so after drinking the beverage. While there were no group differences at baseline or following placebo, figure 4 demonstrates the percent of increase in body sway for the two groups after the low ethanol dose. The FHNs increased their level of body sway significantly more than the FHPs, a finding consistent with the subjective response (Schuckit 1985b). Similar but less intense group differences were again noted after the 1.1 ml/kg of ethanol.

Finally, the levels of two hormones known to change after drinking were observed as indirect indicators of the body's response to alcohol. Serum levels of prolactin (PRL) increased after ethanol administration for both family groups, but returned toward and below baseline more rapidly for the FHPs (Schuckit et al. 1983). Similarly, following the 0.75 ml/kg challenge, plasma levels of cortisol increased for the FHNs but not for the FHPs; and overall, levels of this hormone were lower for the FHPs during most of the experiment (Schuckit 1984d). While hormone levels were only measured after a single ethanol dose and definite conclusions could not be made in the absence of data from a placebo session, the FHP/FHN differences were significant and results are consistent with a less intensive reaction to ethanol for FHPs.

In summary, our laboratory is searching for phenotypic markers of differences between men at higher and lower risk for alcoholism. Once such markers are identified, the next step will be to determine their level of genetic control, the actual mechanisms involved in the phenotypic behaviors, and the level of correlation between the markers and the future development of alcoholism. While the genetic work is in its early stages, one consistent lead involves a decreased reaction to modest doses of ethanol in young men at elevated future risk for this disorder. It is possible that one mechanism of increasing the vulnerability toward alcoholism might occur through a decreased ability to feel the effects of low ethanol doses, perhaps making it more difficult to tell when it is time to stop drinking. The differences in subjective response between FHPs and FHNs have been replicated in two other laboratories (Mednick 1983; O'Malley and Maisto, in press).

These comments were offered as a model of a research strategy that can be used in attempting to identify mediators of a genetic

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influence in substance abuse. Perhaps similar approaches can be adapted to the study of genetics of a vulnerability toward misuse of other substances. However, before that can be done, much work needs to be carried out to establish the importance of genetic factors in the predisposition toward abuse of substances other than alcohol.

FUTURE RECOMMENDATIONS

The information presented here can be used to help develop guidelines for future research in genetics of substance abuse. While this list is not exhaustive, I have attempted to outline factors that are worth consideration.

1. Much work needs to be done to establish whether various forms of substance abuse are genetically influenced. Only after the importance of these genetic factors has been established should studies of populations at high risk for substance problems be carried out.

2. For meaningful research in genetics of substance abuse, it is important that the definition of abuse of substances be carefully considered. Investigators must distinguish between (a) use of drugs, (b) temporary and relatively mild problems associated with occasional drug use, and (c) pervasive and persistent problems that might be best termed substance abuse (Schuckit 1984c). Different genetic factors may contribute at differing levels of importance to each. For instance, genetic factors may have nothing to do with why someone decides to try a substance that is readily available (such as alcohol or marijuana), genetics might contribute slightly to the risk for occasional problems, and an entirely different set of genetic factors could have a major impact on the vulnerability toward more pervasive and persistent problems.

3. It is possible that different genetic influences might contribute to a vulnerability toward the abuse of alcohol than those which impact on the abuse of heroin, and these might be separate from factors involved in a predisposition toward polysubstance abuse.

4. It is important to distinguish between primary substance abuse and problems related to substance abuse that occur only in the midst of other major psychiatric disorders (secondary substance abuse) (Schuckit 1983, 1984c). For example, it is probable that important genetic factors influence the development of the antisocial personality disorder, which in turn often is associated with abuse of multiple substances (Vaillant 1982). Thus, the set of genetic factors associated with substance abuse in patients with a

primary antisocial personality disorder could be different from those associated with substance abuse in primary drug abusers.

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Recommendations for Future Research on Genetic and Biological Markers in Drug Abuse and Alcoholism

Monique C. Braude, Ph.D., and Helen M. Chao, Ph.D.

It is now generally agreed that genetic predisposition plays an important role in the development of alcoholism in some individuals. The participants at the joint NIDA/NIAAA Technical Review on Genetic and Biological Markers in Drug Abuse and Alcoholism reported that there is so far little evidence linking drug abuse to genetic markers. However, the consensus of opinion was that substance abuse could have a genetic component and, like alcohol abuse, more than a single gene could be affected, i.e., it would be polygenic in nature.

The workshop participants were asked to present their ideas for future research which would build upon the current knowledge base. Most of the question and answer sessions following the oral presentation of each paper, as well as most of the discussion session at the end of the meeting, centered on this issue.

It was recommended that a number of steps be taken by both NIDA and NIAAA to share knowledge and to plan future collaborations. It was felt that NIDA should increase its research effort at the preclinical and clinical levels to remedy the paucity of information in this field and to determine whether or not genetic predisposition is a factor in substance abuse.

The following is a synthesis of the highlights of some of their recommendations; it is not intended as, and should not be considered to be, an exhaustive list.

1. Development of genetically defined animal models of alcohol and drug abuse

Much of the empirical base of genetic information related to alcohol and drug abuse has come from animal studies. Experimental animals selected by genetic breeding for various responses to alcohol or drugs are powerful tools for studying biological predisposition. Several lines of rodents have been successfully bred for alcohol-drinking behavior and CNS sensitivity to alcohol. Research along

this avenue should be continued in the alcohol field. Selective breeding approaches should also be used to develop models to study drugs of abuse, to obtain genetic stocks of animals that differ in avidity, in initial sensitivity, tolerance development, and severity of withdrawal for specific drugs of abuse. The development of strains of preferring or nonpreferring animals is usually done in mice or rats (faster and cheaper), but care has to be exercised that the results in rodents can be extrapolated to higher species. This is a long-term approach; it requires considerable amounts of money and expertise, as well as time, but has been very fruitful in the alcohol field.

2. Studies of the genetic makeup of primates resistant to substance abuse

A search could be initiated in the various primate studies supported by NIDA to look for animals which are not vulnerable to substance abuse and to determine their genetic makeup, i.e., to determine which animals are protected and what are the limits of that protection.

3. Screening of inbred strain for differences in the trait of interest

Mice or rat strain differences in response to drugs of abuse have been reported for a variety of drugs of abuse: alcohol, amphetamines, barbiturates, hallucinogens, opiates, and others. Most of these studies have investigated acute responses to drugs, and a few have looked at drug self-administration or severity of a withdrawal syndrome. The fact that strain differences have been identified makes one assume that genetic factors are important in regulating drug abuse responses in animals and that genetic markers might be found. The various preclinical studies looking at different genetic strains should be reoriented towards increased emphasis to the problems of addiction rather than using as biological markers other effects such as motor activity or analgesia which may not be directly relevant to the addiction process. NIDA is already supporting work in this area, and this reorientation should add very little cost to the program.

4. Adoption and twin studies

Adoption and twin studies have provided strong evidence for genetic involvement in the development of alcoholism. Well-designed prospective and longitudinal studies on children of alcoholics should be continued to study inheritance patterns and predictors of familial or clinical subtypes of alcoholism. Studies of families in which detailed clinical data can be correlated to biological and genetic markers need to be conducted. Attention must be given to refinement of subtype classification as well as to the contribution of environmental factors.

In regard to human studies, it is evident that NIDA's program has not yet been fully developed. For instance, there is so far only one small twin study published on the effects of opiates and only one study supported by NIDA on the genetic correlates of marijuana use. Population studies such as those conducted by the alcohol researchers need to be pursued to establish the role of genetics in addiction behavior.

An increased collaboration between NIDA and NIAAA was recommended, as some of the human studies could share protocols and populations. The alcohol researchers attending the meeting mentioned that they would gladly collaborate with NIDA and could insert in their questionnaires items relevant to drug abuse, thus making the human studies less expensive than if initiated by either Institute separately.

5. Genetic markers directly linked to alcoholism and drug abuse

Such markers need to be studied in order to understand the relationship between the specific gene and the addiction process. Studies should be conducted to test major locus effects on susceptibility to alcohol and drug abuse and to assess the contribution of identified major gene loci to such risks.

6. Increased clinical-preclinical communication

As in many other areas, the participants strongly recommended that increased communication be developed between the clinical and preclinical researchers through joint meetings such as workshops or seminars, as it is clear that there is a definite lack of active communication between the clinical and preclinical scientists.

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